RESEARCH BULLETIN NO. 23

THE UNIVERSITY OF NEBRASKA

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OF THE

AGRICULTURAL EXPERIMENT STATION

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Relation of Environment and Other Factors to Potato Wilt Caused by Fusarium Oxysporum

R. W. Goss
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CONTENTS

SUMMARY

1.—*Fusarium oxysporum* is a soil saprophyte capable of parasitizing the potato plant under conditions favorable for the growth of the organism, and the action of the disease after infection has taken place is accelerated by conditions unfavorable for the host.

2.—There are three methods of infection of potato plants with *Fusarium oxysporium* 1st, infection from the soil thru the seed tuber, 2nd, from the soil thru the roots and stem, and 3rd, infection from the seed tuber.

3.—The initial infection is largely influenced by environmental conditions, and the environmental conditions may also cause a considerable change in the latter symptoms of the disease.

4.—The complexity of environmental factors influencing the disease results in many inconclusive results from inoculation experiments and increases the difficulty of interpreting the behavior of the disease in the field.

5.—It was found impossible to consistently reproduce the disease under any of the conditions and with any of the methods used in these experiments.

6.—Growth-temperature studies of *Fusarium oxysporum* in pure culture show that different strains of this species vary in their optimum temperature for growth at least 5°C. These differences were more marked in liquid cultures than in petri dish cultures. It was also shown that by the use of different liquid media it was possible to cause a lowering of 5°-10° in the optimum temperature for growth.

7.—Pathogenicity tests of growing plants showed that the potato plant is more susceptible to infection during its early growth.

8.—The host plant may often be infected without the symptoms of the disease appearing, under conditions favorable for the plant.

9.—A discoloration of the vascular system of both stem and tubers in the absence of any causal organism is often produced under conditions of high temperature and low soil moisture.

10.—Soil infection experiments with pure cultures of the organism under a wide range of conditions, in which the temperature and soil moisture were controlled, showed the usual

small percentage of infection. The results, however, clearly indicated that temperatures of 18°C. and below are very unfavorable for the development of the disease and that the amount of infection increases with increasing soil temperatures. The disease develops most rapidly when the temperatures are too high for vigorous growth of the host plant. Plants started at a temperature of 18° and later changed to higher temperatures showed as much disease as those kept at a constant high temperature. Plants started at a high temperature and later transferred to low temperatures showed practically no disease symptoms even tho the organism was present in small amounts in the finer rootlets.

11.—Constant low soil moisture is unfavorable for infection. After plants have become infected a lowering of the soil moisture accelerates the wilting of the plant.

12.—With increasing soil moistures the amount of rotting of the stems and roots of infected plants increases and the wilting symptoms are not as marked.

13.—In all the infection experiments with inoculated soil, infection took place thru the roots rather than thru the seed-piece.

14.—Field experiments with seed inoculations under three different environmental conditions showed that the greatest amount of disease developed in the plot having low soil temperatures during the early period of growth with a decreasing soil moisture content and increasing soil temperatures in the latter part of the experiment. With conditions very similar except for the application of two irrigations during the latter stages of growth, a small amount of disease developed but the plants were more vigorous. In another plot with the same general type of temperature and moisture curve as in the one showing the greatest amount of disease, but with much higher temperatures and higher soil moistures thruout the experiment, practically no disease developed.

15.—Infection from the soil was found to be the prevalent method of infection in Michigan and Nebraska. Such infection was usually thru the roots rather than thru the seed-piece.

16.—Only a small per cent of tubers having vascular discoloration contain *Fusarium oxysporum* and these tubers do not usually reproduce the disease except under conditions very favorable for this type of infection.

17.—Neither vascular discoloration of the tuber nor of the stem is a good index of infection with *Fusarium oxysporum* unless

these symptoms are found in connection with a wilted plant. Vascular discoloration can be produced in the absence of any organism and can often be correlated in the field with high soil temperatures combined with drought.

18.—Control of the disease by cutting away the discolored portion of seed tubers is not practicable.

19.—Seed tubers showing vascular discoloration, while they usually do not reproduce the disease directly, do produce weak plants that are easily infected from the soil. Such tubers should not be used for seed purposes.

Relation of Environment and Other Factors to Potato Wilt Caused by Fusarium Oxysporum[1]

R. W. Goss

INTRODUCTION

It has been generally accepted by plant pathologists that *Fusarium oxysporum* Schlect. is a very active pathogene causing one of the most widespread and serious diseases of the potato, commonly known as Fusarium wilt or blight. This opinion, however, has been based chiefly upon observations rather than upon conclusive experimental evidence. Many of these observations relating to the widespread activity and seriousness of the disease have been based upon data which have since been proved to be a poor criterion in judging the amount of disease.

A critical review of the literature shows that much of the experimental evidence is either incomplete or negative. Many of the inoculation experiments reported by various investigators were unsuccessful. Results from repetition of the same experiments were often contradictory, and there has been a general failure to reproduce the disease as it occurs in the field. Certain types of the disease have been produced while other types have never been produced experimentally. In most of the inoculation experiments reported in the literature, the methods used have been very drastic and even then a rather low percentage of infected plants has been obtained. The evidence presented does not justify many of the widely drawn conclusions as to the extremely active pathogenic nature of *Fusarium oxysporum*, but rather points out a lack of knowledge of the influence of various factors upon the occurrence of the disease.

In studying potato wilt caused by *Fusarium oxysporum* under widely divergent conditions, it appeared to the writer that there were three probable methods of infection:

1. From the soil thru the seed tuber.
2. From the soil thru the roots and stems.
3. From seed tubers having internal infection.

These three types of infection would be influenced largely by the following factors: 1. Soil temperature. 2. Soil moisture.

[1] This paper is based upon experimental work undertaken at the Michigan Agricultural College in 1914-15, at the University of Wisconsin in 1916-17 and 1920, and continued at the University of Nebraska during 1921-22. The writer wishes to acknowledge his indebtedness to the first two institutions for the privileges and facilities of their laboratories and especially to the members of the Plant Pathology Department of the University of Wisconsin and the Botany Department of the Michigan Agricultural College for their advice, assistance and criticism of this work.

3. Pathogenicity of the strain of *Fusarium oxysporum* present.
4. Temperature differences with different strains of *F. oxysporum*.
5. Differences in susceptibility of the potatoes, both individual and varietal. The disease would also be influenced more indirectly by atmospheric temperature, relative humidity, sunshine, and other factors influencing the development of the host.

This complexity of factors tends to produce uncertain or inconclusive results in experimental work and increases the difficulty of interpreting the behavior of the disease in the field.

The present work was undertaken to determine the influence of some of these environmental factors on the infection and progress of the disease. The work has not progressed to the point of determining the exact relation of each of these chief factors. However, the results thus far obtained are presented here in order to direct attention to the nature and distribution of the disease in relation to the environmental condtions under which certain types of infection take place.

PREVALENCE AND LOSS

It is impossible to determine accurately the actual loss in yield due to *Fusarium oxysporum*. The nature of the disease is such that an estimate of the loss in commercial fields is a matter of guesswork rather than of accurately compiled data. It is true of this disease, as of many others, that the estimated losses are often exaggerated, owing to confusion with other troubles, and to the fact that actual losses in yields cannot be accurately estimated except in experimental plots or in cases of epidemics.

The distribution of the disease is widespread, as the organism is almost universally present in the soil. Pratt (1916)[1] found that disease-free tubers planted on virgin desert soil in Idaho produced 29.3 per cent vascular infection as determined by pure cultures. Werkenthin (1916) found *Fusarium oxysporum* to be present in virgin soils of Texas. The writer has isolated the organism from virgin forest soils in the upper peninsula of Michigan, and from fields having been in sod for over 20 years.

No doubt in certain localities the disease has approached epidemic proportions in some years. However, the estimated losses in the United States for three years, as given by the Plant Disease Survey (1919-20-21), show a rather small amount of damage.

[1] All references to literature are indicated in the text by the name of the author and the year of publication. For full citations, see the list at the end of this bulletin. Reviews of individual papers are divided into various sections to conform to the outline of this paper.

Year	Loss per state Per cent	No. states with 10 per cent loss	No. states with 5 per cent loss
1918	2.3	5	14
1919	1.6	2	7
1920	1.6	2	4

In the six leading states in potato production for these three years, the highest reported losses are 6 and 5 per cent in 1918. In 1919 and 1920 none of these states report more than 3 per cent loss.

Many estimates of the amount of infection have been based upon vascular discoloration rather than upon the number of wilted plants in the field, altho we are now aware that vascular discoloration is not a true index of the presence of *Fusarium oxysporum*.

In 1914, 100 fields in Michigan being grown for seed purposes were inspected by the author. The amount of disease was estimated both by counts of the number of wilted plants and by digging up 4 or 5 lots of 10 hills each in every field and examining for vascular discoloration. Twenty-one per cent of the fields showed Fusarium wilt as evidenced by actual wilting; only one of these fields showed more than 10 per cent infection, and the average amount of infection was 1.5 per cent. If the vascular browning of stems or tubers had been used as an indication of disease, it would have been found that out of the 100 fields inspected, 96 showed plants with discolored vascular systems, averaging 30 per cent infection per field.

In Nebraska, in 1920, the author examined about 900 acres and found about 82 per cent of the fields infected with wilt, averaging 6 per cent infection. In 1921 as a result of the seed certification work carried on by the Horticultural Department of the University of Nebraska, 83 per cent of the acreage inspected was found to contain wilt averaging about 3 per cent infection. These estimates in Nebraska also include a considerable amount of wilt due to causes other than *Fusarium oxysporum*, so that the actual damage due to this one organism is considerably less than the above reports would indicate.

Judging from the results of a number of surveys made in different states, the author considers Fusarium wilt to be a widespread disease which in some sections may produce considerable injury over limited areas but is usually not serious over entire states. The wide variations in the amount of infection in some states over a period of years indicate that environmental conditions play an important role in determining the severity of the attack. The amount of infection in the same fields in successive

years will vary widely, irrespective of the potatoes planted or treatment of either soil or seed.

SOURCE OF CULTURES

The various strains of *Fusarium oxysporum* used in all the following experiments are listed below with their origin.

Strain No. 1.—Isolated by the author from the discolored vascular bundles of a potato tuber in Michigan, October 15, 1914. This culture was identified by H. W. Wollenweber and numbered 3377 by the United States Department of Agriculture.

Strain No. 8.—Culture obtained from C. W. Carpenter of the United States Department of Agriculture as No. 3395.

Strain No. 32.—Culture obtained from G. K. K. Link of the University of Nebraska as No. 3345a, and obtained by him from the United States Department of Agriculture.

Strain No. 33.—Culture received by James Johnson of the University of Wisconsin from W. G. Gilbert, United States Department of Agriculture as No. II.

Strain No. 35.—Reisolation of Strain No. 33 made in 1920 from inoculated plants showing wilt.

Strain No. 56.—Reisolation of Strain No. 33 made in 1921 from the roots of inoculated plants.

While these strains were of greatly different ages, no differences could be observed in any of the inoculation experiments indicating a loss in virulence due to the age of the culture.

GROWTH-TEMPERATURE RELATIONS OF FUSARIUM OXYSPORUM IN PURE CULTURE

In studying the relation of temperature to the infection and progress of the disease it was also deemed advisable to determine the effect of temperature on the growth of the organism in pure culture. While such a study does not necessarily give a true index of disease phenomena under natural conditions, it does aid materially in interpreting the action of the disease both in the field and under controlled experimental conditions.

In the literature dealing with the growth-temperature relations of *Fusarium oxysporum* in pure cultures there is a general uniformity in the type of growth curve. However, the exact minima, optima, and maxima for growth, each vary in the different papers from 5° to 6° C. No attempt has been made in the literature to explain this discrepancy in the results of various workers.

In reviewing the literature on the subject it was found that in all the experiments reported it was assumed that different strains of *Fusarium oxysporum* would react similarly to the same temperature conditions: that is, while the total growth might vary, the cardinal points for growth would remain the same. It was also assumed that the same effect upon the growth-temperature relations would be true of the use of different media.

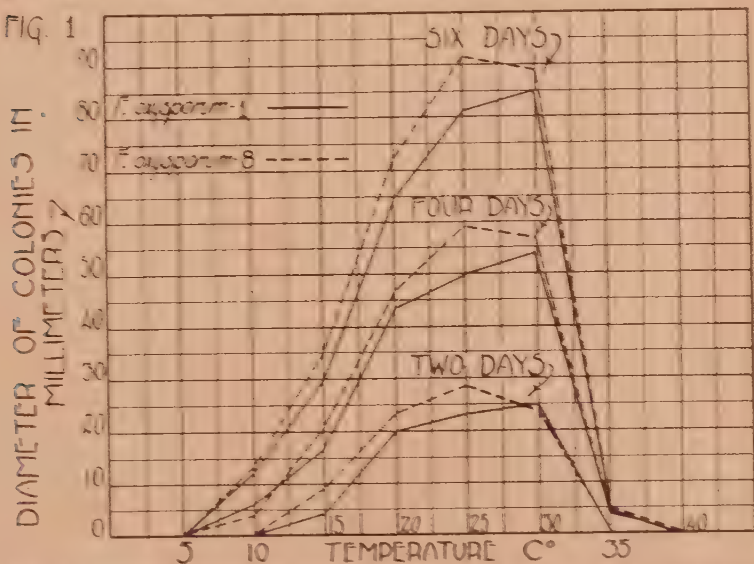


FIG. 1.—Graph showing the rate of growth of 2 strains of *Fusarium oxysporum* on potato agar at different temperatures.

Preliminary experiments conducted by the writer indicated that different strains react differently to temperature effects. The media used were also found to cause a shifting of the cardinal points for growth. Further experiments were therefore conducted with a number of strains of *Fusarium oxysporum* and with several other species of Fusarium. These tests were made on various media both solid and liquid.[1] While lack of space will not permit the publication of the detailed experiments at this time, typical examples of some of the results are presented here.

In test tube cultures of Strains Nos. 1, 8, and 32 on potato plugs, submitted to temperatures between 5° and 38° at about 5° intervals, it was found that growth occurred at 9.5° but not at 7°; growth also occurred at 37.5° but not at 38°C. The

[1] These results are being published as a separate paper.

optimum temperature for growth was between 24° and 28°. No differences in the reaction of the different strains to temperature were noted.

In petri dish cultures on hard potato agar it was found that different strains produced their maximum growth at different temperatures. In these cultures the daily increase in the diameter of the colony was used as the index of growth. Figure 1 shows the comparative results of 2 strains of *Fusarium oxysporum*. It is evident that one strain shows greater growth at the lower temperatures and its maximum growth is produced at a point 5°C. lower than the other strain. The difference was found in all trials and in all media, being even more marked in liquid media.

FIG 2

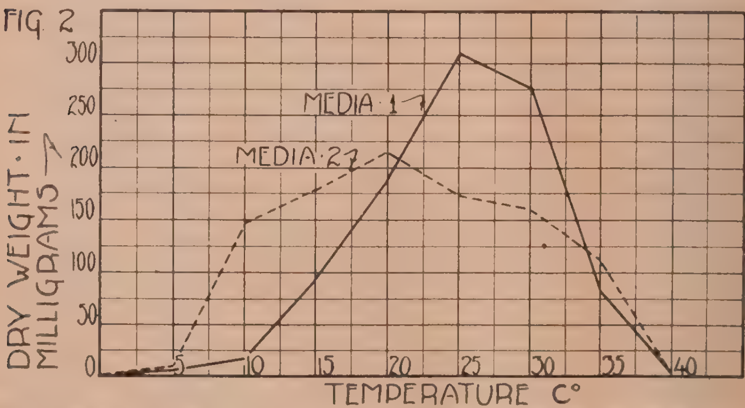


FIG. 2.—Graph showing the comparative growth of *Fusarium oxysporum*, after 3 weeks on 2 different liquid media.

In tests with liquid media, determining the growth by dry weights, it was also found that the use of different nutrient solutions resulted in a shifting of the minima, optima, and maxima temperatures for growth. Figure 2 shows the average results of 4 trials with *Fusarium oxysporum* No. 32. In these tests the 2 media used were made up as follows:

Media No. 1	Media No. 2
125 c.c. M/1 NH_4NO_3	20 gm. NH_4NO_3
50 c.c. M/1 KH_2PO_4	10 gm. KH_2PO_4
20 c.c. M/1 Mg SO_4	5 gm. Mg SO_4
150 c.c. M/1 Sucrose	500 c.c. Potato extract from 500 gm. of potatoes.
1 c.c. M/1000 Fe Cl_3	
654 c.c. Distilled water	500 c.c. Water
1000 c.c.	1000 c.c.

It is evident that these 2 media cause a considerable difference in the reaction of the same organisms to different temperatures. In Media No. 2 there is a shifting of the optimum temperature for growth 5°C. below that for Media No. 1. There is also a greater growth at the suboptimal temperatures and less growth in the supraoptimal region. This difference was also found with a number of other strains and species.

In liquid media it was also possible to obtain weighable growth at 5°C., while in petri dish cultures no growth was observed at this temperature.

These data explain to some extent the different results reported by various investigators. They also show that in inoculation experiments with controlled temperatures the manifestation of the disease at certain temperatures may depend partially at least upon the strain of organism being studied.

INFECTION EXPERIMENTS

It has always been recognized that Fusarium wilt is very greatly influenced by environmental conditions, chiefly by the temperature and moisture of both soil and air. Just how great a part these factors play in determining the infection and progress of the disease has not previously been determined experimentally.

In the following work presented in this paper an attempt has been made to study this disease with various factors under control. The chief difficulties lie in the fact that at present we must of necessity work with maintained conditions rather than with the fluctuating conditions found in nature. The number of individuals in each test is very small under the best experimental conditions and our knowledge of the reactions of the host to the various environmental conditions even in the absence of disease is fragmentary. The results, as to the effect of the various environmental factors, are presented separately for the different types of infection.

Pathogenicity Tests By Direct Inoculation of Growing Plants

Altho it has been generally accepted that *Fusarium oxysporum* is the causal organism producing the wilt of the potato vine, until recent years there have been practically no inoculation experiments reported, and even in the few experiments reported the results have often been inconclusive.

REVIEW

Smith and Swingle (1904) isolated the organism and described the disease, but no inoculation experiments were reported.

Manns (1911) reported inoculation experiments but he does not give any detail experiments with pure cultures. He states (p. 316):

> Artificial infections were brought about in a number of ways. Slight injuries of the roots or knife incisions in the presence of the organism brought about the most rapid infections. The organism is, however, productive of infection in the absence of any root disturbance or stem injury, as was shown by a number of experiments.

Link (1916) reports experimental infections of living plants with *Fusarium oxysporum*. He obtained his largest percentage of infections by smearing the sprouts with rice cultures of the organism as they were just breaking thru the ground. By using inoculated soil he obtained variable results and suggests that this may have been due to a loss in vitality of the organism, or to a gain in the resistance of the plant as the season progressed. Sherbakoff (1915) reports that he was unable to obtain positive infection with cultures of *F. oxysporum*. He suggests that this may have been due to a loss in virulence of the cultures.

These few reported experiments with direct inoculations of growing plants have certainly not produced any very definite or conclusive evidence that *Fusarium oxysporum* is a very active wilt parasite. The inoculations while very drastic in their nature, did not always yield uniform results. The evidence indicates that the organism, instead of being a very active parasite, is rather a weak one and that it is probably greatly influenced by conditions that were not kept under control in these tests.

EXPERIMENTAL DATA

The following infection experiments relate only to artificial inoculations of stems and roots, somewhat similar to those reviewed above.

Methods.—In the following experiments only vigorous plants grown from healthy seed were used. The tubers were examined for the absence of any vascular browning, treated with mercuric chloride and planted in sterilized soil. In all cases unless otherwise stated, the stems were inoculated by removing the soil from around the stem, which was then disinfected, and a small wound made with a sterilized scalpel just below the surface of the ground. A small amount of the inoculum was then introduced into the wound and the soil packed back in position and a little sterile water added.

Greenhouse Experiments

EXPERIMENT 1, MARCH, 1916

Plants of the Rural New Yorker variety growing in the greenhouse were inoculated at different ages with different strains of *Fusarium oxysporum* in an attempt to observe any differences in the virulence of the strains or any indications of the plants showing different degrees of resistance as they became older.

Set 1. Three plants were inoculated when 2 to 5 inches in height with *Fusarium oxysporum* No. 1, grown on steamed potato cylinders. Plant 1 showed typical wilt and heavy infection throughout the vascular system. In plant 2 the mycelium of the fungus could be seen penetrating the vascular system of the stem for a short distance from the point of inoculation; no wilting occurred in this case. Plant 3 remained healthy.

Set 2. Three plants were inoculated at the same age as those in Set 1 with *Fusarium oxysporum* No. 8. Plant 1 showed a heavy infection and early wilting. Plants 2 and 3 showed only a slight penetration of the host and did not wilt.

Set 3. Three plants 5 to 10 inches in height were inoculated with *Fusarium oxysporum* No. 1. None of these plants showed any symptoms of wilt and only in 2 instances was there a slight penetration of the host from the point of inoculation.

Set 4. Three plants 5 to 10 inches in height were inoculated with *Fusarium oxysporum* No. 8. No wilting occurred. All plants showed a slight vascular discoloration for about 1 inch from the point of inoculation.

Set 5. Six plants used as controls remained healthy except in one instance where a wilting of the tops occurred and from which *Fusarium oxysporum* was isolated.

These few tests do not show consistent results, but do indicate that the inoculation of younger plants will give a higher percentage of infection. There was no apparent difference in the 2 strains of *Fusarium oxysporum* used.

EXPERIMENT 2, MARCH, 1917

Four plants of the Rural New Yorker variety growing in the greenhouse were inoculated, just as the shoots were showing at the surface of the ground, with *Fusarium oxysporum* No. 8. All 4 of these remained healthy.

The previous test was repeated. Seed from the same source was used and inoculations were made with the same strain of *Fusarium oxysporum* just as the sprouts broke thru the ground. Three of these plants showed early signs of wilt. The fungus was present abundantly in the vascular tissue, and was recovered in every instance in pure culture. One plant remained healthy. All control plants remained healthy.

EXPERIMENT 3, FEBRUARY 21, 1920

This experiment was conducted at two different temperatures, half of the plants being placed in a greenhouse maintained at 17°, and half in another house at 27°C. In each house 5 plants were inoculated when they were 10 days old and 4 others were used as controls. Some of the plants at each temperature were inoculated with *Fusarium oxysporum* No. 33, and some with No. 8.

Results.—17°C. The plants were all examined May 6. Two plants were inoculated with *Fusarium oxysporum* No. 33. One stem of plant 1 was inoculated; there was no infection and the wound calloused over; 4 control stems remained healthy. One stem of plant 2 was inoculated, no wilt resulted, the wound calloused over and there was a slight penetration of the vascular system for ¼ inch beyond wound; 2 control stems remained healthy. Three plants were inoculated with *Fusarium oxy-*

sporum No. 8. All wounds healed over and there was no infection or sign of wilt. All controls remained healthy.

Results.—27°C. Four plants were inoculated with *Fusarium oxysporum* No. 33. Three stems of plant 1 were inoculated; the tops wilted slightly, the wound on the stem healed over and there were diffuse light brown lesions around the stem but no rotting. Sections of the stem showed invasion of the vascular system and browning extending ¾ inch above the wound. *F. oxysporum* was recovered from the vascular system. Plant 2 remained healthy; the wound healed over, and there were no lesions or browning of the vascular system. Infection did not progress beyond the wound. Two stems of plant 3 were inoculated, there was no wilt but the organism invaded the vascular system for 1 inch above the wound and below as far as the roots. No external lesions were present. *F. oxysporum* was recovered. Three stems of plant 4 were inoculated. One stem with healthy tops showed a slight invasion of the tissue around the wound, while 2 other stems with the tops completely wilted had the underground stem entirely rotted below the wound. One plant was inoculated with Strain No. 8. There was no infection; the entire plant remained healthy, and the wound healed over. The controls remained healthy.

EXPERIMENT 4, 1921-22

During the winter of 1921-22 a few inoculations were made on plants in the greenhouse. Tubers of the Bliss Triumph variety were planted in tuber units; 1 plant was used as a control and 3 others were inoculated, both by stem inoculations and by pouring a water suspension of spores on the soil at the same time. The plants were inoculated when they were 6 to 8 inches high.

Three inoculated plants and 1 control were kept in a glass incubating chamber where the soil and air temperature was kept between 30° and 36°C. The other set of 4 plants was kept at a temperature of about 24°. The plants were dug and examined 6 weeks after inoculation. In the set at 30° to 36°, plant 1 had 1 stem entirely dead and the other slightly yellowed. Plant 2 was entirely dead, apparently due to temperature. Plant 3 wilted completely. The control plant was also completely wilted. In the set at 21° to 26° all plants showed a slight yellowing of the foliage; there was no complete wilt and no discoloration of the vascular system. The control plant remained healthy.

EXPERIMENT 5, DECEMBER, 1921

Inoculation tests with other organisms to determine the relative values of the methods of inoculation were made in 1921. Cultures of *Penicillium* sp. and *Rhizopus* sp. were used in order to test whether the methods generally used in determining the parasitism of *Fusarium oxysporum* are so drastic that even very weak parasites would be able to produce similar symptoms.

Potato plants of the Bliss Triumph variety grown in the greenhouse at about 24°C. were inoculated either by smearing the emerging sprout or by inserting into the stem portions of agar cultures of the organisms to be tested. The plants were dug up and examined one month after being inoculated.

Results.—No wilting occurred in any of the plants. Plants inoculated with *Penicillium* sp. or *Rhizopus* sp. by smearing the emerging sprout remained healthy and the organisms did not penetrate the host

tissue. When the sprouts were wounded and the inoculum inserted it was found that both organisms had penetrated from 1/4 to 1½ inches beyond the point of inoculation. The vascular tissue was discolored, but the infection was not entirely confined to vascular tissue. *Rhizopus* sp. was able to cause considerable more rotting than *Penicillium* sp. and produced symptoms very similar to those found in the inoculations with *Fusarium oxysporum.*

Field Experiments

EXPERIMENT 1, 1916

Methods.—Inoculation experiments with pure cultures were made on a large scale in the field in the summer of 1916 in Michigan. Potato plants of the Rural New Yorker variety grown from healthy seed were used for this experiment. Some of these plants were inoculated as soon as the sprouts showed thru the ground, others when they were 5 to 6 inches high. Inoculations were made either by introducing the organism into the stem just below the surface of the ground or by soil inoculation both with and without wounding the roots. The inoculum was obtained from 10-day-old potato plug cultures of *Fusarium oxysporum*, also with three varieties of *F. oxysporum* obtained in 1915 from C. D. Sherbakoff at Cornell University. Altogether, about 1,000 plants were inoculated. The tubers were planted June 14 and 15, the sprouts were inoculated the last week in June, and the final results taken at digging time, September 10.

During the experiment air temperature records were taken by means of a thermograph placed in a shelter in the center of the field. Soil temperature records were taken with maximum and minimum thermometers placed in the soil at the depth of 5 inches and readings taken every 24 hours. After August 15, a soil thermograph was used. Precipitation records were obtained from the United States Weather Bureau located about one mile away, and these give a fair indication of the moisture content.

The air temperature was very high during the months of July and August. The mean maximum temperature for July was 91.8° (33.2° C.) and for August 84.9° F. (29.4° C.). The mean average temperature for July was 71.1° (21.7° C.) and for August 72.7° (22.6° C.). The highest recorded temperature was 104° (40° C.) the 29th of August. It is worthy of notice that from the 20th of July to the 7th of August the lowest maximum temperature was 92° (33.3° C.). The soil temperature from July 20 to August 15 showed a mean maximum of 104° (40° C.) and a mean minimum of 63° (17.2° C.). The highest temperature, 111° (43.9° C.), was reached a number of times and was the highest that the only available thermometers at the time would record. Undoubtedly the temperature went higher than this on a number of days. After August 15, the soil temperature dropped considerably, owing to an increase in the rainfall. The total precipitation for July was only .09 inch and for August 1.58 inches.

Results.—No typical wilting occurred in any of the thousand-odd plants inoculated. Each hill was examined for infection by cutting sections thru the root and tubers, and also thru the stem. More than 90 per cent of the inoculated plants were found to have a slight browning of the vascular system, and the controls contained a similar amount. A large number of cultures were made in the laboratory in an attempt to isolate the organism from these discolored vascular areas, but without exception they were unsuccessful.

The combination of very low precipitation and very high

temperature during this experiment produced an environmental condition very unfavorable for the growth of the potato. Furthermore, these conditions were also unfavorable for infection by *Fusarium oxysporum* even tho Fusarium wilt is usually considered a warm weather disease. It is evident that the low soil moisture content was not favorable for initial infection and therefore high temperatures combined with low moisture have no influence on the disease unless the moisture content has previously been high enough to permit the initial penetration by the causal organism.

EXPERIMENT 2, 1921

During the summer of 1921 a few inoculations of growing plants were made in the experimental potato fields at Lincoln and Mitchell,[1] Nebraska. In the Mitchell fields the experiments were conducted under both dry land and irrigated conditions. The air and soil temperature and moisture records are shown in Figures 3, 4, and 5. Healthy tubers of the Bliss Triumph variety planted in tuber units were used for these tests.

Plot 1, Lincoln.—Tubers were planted June 4 and were inoculated June 25. Stem inoculations were made the same as in previous experiments, every stem in each hill being inoculated. The plants were 4 to 5 inches high at the time of inoculation. Four plants were inoculated with *Fusarium oxysporum* No. 1, 4 with No. 8, 6 with No. 35, and 8 with No. 56. One hill in each tuber unit was used as a control.

Results.—None of the plants at any time showed symptoms of wilting. Both inoculated and control plants were small and considerably dwarfed, due to the high temperature of both soil and air. The plants were dug September 2. All inoculated plants showed a browning of the vascular system from the point of inoculation to the base of the stem, while the control plants remained healthy.

Plot 2, Dry Land, Mitchell.—The tubers were planted May 20. Inoculations were made June 17, when the plants were from 1 to 6 inches in height. Stem inoculations were made in the same way as the previous experiments. Five plants were inoculated with *Fusarium oxysporum* No. 35 and 5 with No. 56.

Results.—On August 16 one plant inoculated with Strain No. 35 showed typical Fusarium wilt. All other inoculated plants and controls remained healthy. These plants were dug September 21. Of the 4 remaining plants inoculated with No. 35, 1 was perfectly healthy and 3 showed a browning of the vascular system of the stem. One of these 3 produced tubers showing vascular browning. Of the 5 plants inoculated with No. 56, 2 remained healthy. One plant showed vascular browning of the stem and produced 1 tuber out of 6 showing vascular browning.

Plot 3, Irrigation, Mitchell.—Tubers of the same lot as those in Plot 2 were planted and inoculated at the same time. The plants averaged about 4 inches in height when inoculated. Six plants were inoculated with *Fusarium oxysporum* No. 35 and 6 with No. 56.

Results.—No wilting occurred in any of the 12 inoculated plants, altho when dug September 21, practically every stem showed considerable

[1] Scotts Bluff Substation, located at Mitchell, Nebraska.

vascular browning; no rotting of the stem was present and no infected tubers were produced.

DISCUSSION

These preliminary tests of the pathogenicity of *Fusarium oxysporum* made in a similar way to those reported in various papers on this disease show very clearly that consistent results are not obtained by these methods even tho the methods of inoculation are drastic enough to permit similar symptoms to appear when inoculations are made with relatively weak parasites. The large number of negative results indicates clearly that *F. oxysporum* is not a very virulent or a very destructive organism. These tests also show that the plant is more susceptible to infection during the early growth. It is also shown that considerable tissue may be infected and the vascular system distinctly discolored without any symptoms of wilt appearing on the plant. The experiments in the greenhouse indicated that high temperatures favor the development of the disease. However, the field experiments conducted in 1916 show clearly that an extremely high temperature and a very low soil moisture content produce conditions unfavorable for infection. Under these conditions a discoloration of the vascular system occurs that is not connected in any way with the disease.

TRANSMISSION OF FUSARIUM WILT BY INFECTED SEED

Review of the Literature

VASCULAR DISCOLORATION OF TUBERS

Until very recently it has been commonly believed, as evidenced by the oft repeated assertions found in bulletins, circulars, and in practically all the information given to the farmers relative to Fusarium wilt of potatoes, that a plant infected with Fusarium wilt will produce tubers showing a brownish discoloration of the vascular system, and that a tuber showing vascular browning was produced by an infected plant; further, that a tuber showing such vascular discoloration will, if planted, produce infected plants.

Control measures based upon this hypothesis have been recommended extensively in advising remedial measures to the grower for the control of Fusarium wilt. This method of control appears very simple, i. e., the elimination of infected tubers by discarding entire tubers or cutting away the discolored portions of infected tubers. In recent years a number of papers have been published that do not agree with the earlier reports. Never-

theless, the popular bulletins on potato diseases still contain this information. Inasmuch as there is this conflict of data and also no published review of the literature, it was thought worth while to present a brief review of the various papers presenting experimental evidence on this point.

Smith and Swingle (1904) were probably the first to draw this conclusion and to recommend such control measures. They state (p. 59):

> The first symptoms in the tuber are nearly always at the stem end in the form of browned or blackened vascular bundles.

They also report that cultures made from the discolored vascular bundles very seldom failed to develop the fungus. As regards control measures, they recommend (p. 60):

> The greatest care should be taken to avoid the infection of healthy land by the planting of diseased tubers. All tubers designed for seed should be cut at the stem end and carefully inspected before planting.

They do not, however, give any detailed experiments in which tubers showing vascular browning and from which the causal organism had been isolated were planted and resulted in wilted plants.

Manns (1911) reports field experiments in which seed having vascular discoloration was planted after various treatments, and compared with healthy seed of the same variety. It is unfortunate that the results are chiefly based upon yield in bushels rather than upon the percentage of diseased plants. He concludes from his experiments that the percentage of increase in yield obtained from cutting away the infected portion of the seed combined with formaldehyde treatment varied with the amount of infection in the seed from --5 per cent to +42 per cent. His results (Table 5, p. 327) show considerable variation. One of the plots shows a decrease in yield when the infected portion of the seed-piece is cut away; others show variable increases in yields. It is noticeable that in the plot showing the greatest increase, 42.26 per cent, the total yield is only about 50 bushels per acre, indicating that there were probably other factors present not under control that were materially reducing the yield. In many instances the variation in yield between plots with the same treatment was as great as the increased yields claimed for the treatment, showing a considerable experimental error. It is unfortunate that while the different experiments were conducted in 5 different counties, no records of environmental conditions are given, it is very possible that the environmental conditions would influence the amount of the disease and the average yields as much as the amount of infection supposedly present in the seed

used. It must be remembered that in all these tests we have to do with tubers showing internal discoloration and not necessarily with *Fusarium oxysporum*, as no isolations were reported from the seed tubers. He also notes (p. 325) in one experiment that:

> Fusarium symptoms showed up distinctly on 71.1 per cent of the vines, six weeks before maturity, while the non-infected plot showed only 4.7 per cent of the vines infected.

And again, (p. 328) he reports 76 per cent of tubers showing internal infection when grown from infected seed against less than 1 per cent in tubers from seed in which the infection had been cut away. As a result of his work, Manns concludes (p. 336):

> Internally infected tubers are the chief means of distributing the disease * * * * The infection may be removed from slightly infected seed by clipping away the stem end and following by external treatment with formaldehyde.

Link (1916, p. 188), referring to this method of infection, states:

> Infection carried over by the mother tuber, which is frequent, rarely permits the growth of stems more than 20 cm. high, and seldom allows the development of tubers.

He does not present any experimental data to substantiate this point. If this statement is correct, we should expect that in such experiments as those described by Manns there would be a more marked difference in yield; in fact, we could hardly expect any yield at all.

As far as the author is aware, Bisby (1919) was the first to submit experimental evidence showing that seed having vascular discoloration and having been obtained from wilted vines often produced normal plants. His data (p. 14) indicate that—

> serious disease does not necessarily follow from planting seed from wilted vines, and that no more wilt may result from the use of stem ends than from the use of eye ends.

He mentions, however, the general undesirability of seed from wilted plants and further states (p. 14):

> Selecting seed or clipping the stem ends is, however, not alone sufficient to avoid loss from wilt.

His experiments while not as extensive as those of Manns (1911) were accompanied by some isolations from the seed to determine the presence of *Fusarium oxysporum* and his results are clear-cut.

Haskell (1919), working in New York, states:

> Fusarium oxysporum is undoubtedly disseminated by means of the seed tuber.

However, he immediately qualifies this statement in the following sentence by saying:

When affected potatoes are planted the plants arising therefrom may be diseased provided the proper conditions exist.

He reports experimental plantings of seed showing distinct stem end browning compared with healthy seed with no vascular discoloration. He bases his results on comparative yields and per cent of diseased seed in the resulting crop, and not on percentage of wilted vines. His results show that the infected seed yielded only 9.6 oz. per hill against 13.9 oz. for healthy seed. No data are given as to the size of the experimental plots or total yield. He also found that in the resulting crop 37 per cent of the tubers from infected seed were diseased against 42 per cent from the healthy seed. He attributes this large amount of infection in the check plot to the fact that infection occurred from the soil. He also reports some experiments with a small number of tubers from which in no case were there 50 per cent of the resulting plants showing disease. He does not mention any control plantings but nevertheless concludes (p. 240) from these experiments that:

They show that the causal factor may be communicated to some extent at least through the seed tubers. On the other hand diseased seed often gives rise to plants that produce a healthy crop. This may be either because those tubers do not harbor the pathogene or because it is present but conditions are unfavorable for sufficient development.

He agrees with Manns (1911) and Bisby (1919), however, in concluding that affected seed results in a lowering of the yield. He advises against the planting of seed containing over 5 per cent of "Fusarium necrosis." Seed having less than this amount can be used providing badly diseased tubers are removed either by "greening" and selection for strong germinating power or by cutting the stem ends and discarding diseased tubers.

MacMillan (1919), studying the disease under irrigated conditions in Colorado, states in his summary (p. 301):

Vascular infection of the seed is not the starting point of disease, but is one of the conditions assisting in bringing about decreased resistance to new infection from the soil.

His experiments are more extensive, especially as to the inspection of individual seed-pieces and cultural studies, than any of the above reviewed work. The results are more clear-cut and are given in percentages of diseased plants rather than in yield. He conducted several experiments where seed having vascular infection and from which Fusarium sp. had been isolated,

yielded results which would support his conclusions stated above. He divided his tubers in half, and the records of the twins are given from notes taken 4 times during the summer and summarized under 6 different headings, indicating the absence or presence and severity of the disease. There are comparatively few cases in which 2 hills from the same seed-pieces produced similar symptoms of disease, and this percentage was no greater than that found in hills from healthy seed.

He reports experiments where seed tubers of the Early Ohio variety obtained from wilted plants showing vascular discoloration and yielding Fusarium sp. in culture showed less disease than the plots planted with supposedly healthy seed. He found that seed of the Pearl variety obtained from wilted hills produced a larger percentage of hills with no germination or seed-piece rotting, and the author accounts for this by stating (p. 294):

> Plainly these plants have been weakened by disease, their power of resistance lessened, and their vigor impaired. Examination showed that soil infection acted here to produce the disease.

It is true that while cultures were made in many cases from diseased seed, the organisms recovered were simply classed as Fusarium sp., which does not prove that *Fusarium oxysporum* was the only organism being dealt with. In general, however, it can be said that these results distinctly disagree with many previous publications and show clearly that, under the conditions of the experiment at least, Fusarium wilt carried over in the seed-piece is not an important factor in transmitting the disease. It is also shown that seed obtained from wilted hills will produce weaker plants that are more susceptible to soil infection.

Edson (1920), studying the relation of vascular discoloration of the seed to the disease, presents the most extensive experiments recorded to date on the relation of vascular discoloration to the presence of a causal organism and to the resulting disease in the plants. He used tubers obtained from 19 different sources and divides these into 3 groups, obscure disease group, healthy group, and parasitic disease group. Cultures were made from seed tubers to determine the presence or absence of organisms, and these tubers were planted in 2 lots, 1 in Wisconsin and the other in Colorado. Results are presented in number and percentage of diseased plants. He finds that vascular discoloration is often present in the absence of any organism, and that organisms are frequently present when the tissue appears healthy; out of 3,203 cultures, only 1,851 revealed the presence of organisms and of these Fusarium sp. were present only 720 times. His field experiments indicate (p. 294)—

that neither vascular discoloration nor fungus invasion of the tissues of the mother tuber is a guarantee of disease in the resulting plants, nor is their absence a guarantee of health.

He believes the soil to be the chief source of infection. Detailed results are given in a comparison of the plantings at Wisconsin and Colorado. In general there was more disease present in the Colorado plots than in those at Wisconsin, particularly when cut seed was used. He also notes that the plants were often capable of recovery from disease, particularly in Colorado. While these experiments afforded an excellent opportunity for studying the effects of environmental conditions on the progress of the disease, no detailed information as to the environmental conditions under which the experiments were conducted is given except where it is stated (p. 279) that –

one lot being planted on a light, sandy soil, under rainfall, at Waupaca, Wis., and the other on a heavy clay loam under irrigation at Greeley, Colo.

There is no detailed discussion given on the results of these different environmental conditions other than that stated above. While the results show clearly that the soil rather than the seed-piece is the chief source of infection, it also appears from a study of the data that diseased seed results in a larger percentage of infected plants from soil infection. This is shown clearly by a comparison of the three different groups. The "healthy group" compared with the "parasitic disease group" shows a small percentage of diseased plants for both cut and whole seed and under both Wisconsin and Colorado conditions, regardless of the fact that the healthy seed showed nearly as much vascular discoloration (about 42 per cent) as compared with 44 per cent for the parasitic group. As regards the percentage of cultures showing growth, there was 76 per cent for the healthy and only 72 per cent for the parasitic group, and about 25 per cent of the organisms isolated in both cases were either *Fusarium oxysporum* or Fusarium sp. exclusive of *F. discolor var. sulphurem* (Schlect.) App. and Wollenw. Thus, the amount of infection that could have been carried over would have been about the same in both groups, yet the parasitic group yielded about 10 per cent more diseased plants than the healthy group, this being especially true in the case of whole seed. It is worthy of note that in the parasitic group, more diseased plants resulted from whole seed than from cut seed, while the reverse is ture for the healthy group for both Wisconsin and Colorado plots.

As regards the "obscure disease group," he states that a large amount of hereditary disease was present in this group,

and this is clearly shown from the tables presented. The group shows the smallest number of tubers having vascular discoloration, the lowest percentage of cultures giving growth, and a very low percentage, about 6 per cent, of these positive cultures yielding *Fusarium oxysporum* or Fusarium sp.; and yet the largest percentage of diseased plants is found in this group, being about double that of the parasitic group. Here, again, more disease is found in plants from whole seed than from cut seed. Edson concludes (p. 294) that:

> Stem-end seed-pieces yielded slightly higher percentages of disease than eye-end pieces, evidently because the stem end is endowed with less physiological resistance.

McKay (1921), studying some wilt diseases of potatoes in Oregon, also takes up the question of the occurrence of *Fusarium oxysporum* in discolored tubers and the amount of disease resulting therefrom and draws conclusions similar to those of Edson (1920) and MacMillan (1919). The results of his isolations from tubers showing vascular discoloration show clearly that this factor cannot be relied on as a criterion in judging the presence of *F. oxysporum*. He isolated *F. oxysporum* from 295 tubers, or 2.4 percent of all the tubers cultured. His results also indicated that *F. oxysporum* is associated with a darker discoloration of the vascular tissues than either *F. radicicola* Wollenw., or *Verticillium albo-atrum* Reinke and Berthold. His field experiments also show that *F. oxysporum* is transmitted to only a slight extent thru seed tubers. His results are given, not in yields or percentage of diseased plants, but in percentage of resulting tubers yielding the organism. He obtained from seed known to be infected with *F. oxysporum* only 3.8 per cent of *F. oxysporum* infected tubers, while he obtained 1.6 per cent from seed yielding no organisms and 3.5 per cent from seed yielding *F. radicicola*, an organism that is not known to produce a wilt. He also found that stem-end seed-pieces of infected tubers do not seem to give any more disease than the eye ends of the same tubers. He concludes that the difference in eye end and stem end is so slight that it is not advisable to rely on the practice of discarding the stem ends to avoid wilt. Altho no data are given on the number of wilted plants, his results taken from cultures obtained from the tubers produced would not indicate as great a difference in susceptibility to disease of eye and stem-end seed-pieces as was reported by Edson (1920).

Thus we find considerable discrepancy, not only in the opinion of various workers but also in their experimental data. The preponderance of careful experimental evidence favors the

view that vascular discoloration in the tuber is not always a good index of the presence of *Fusarium oxysporum*, or of the fact that disease will result even tho the organism is present. Nevertheless, such discolored seed appears more susceptible to infection from outside sources. The experimental evidence is rather conflicting in regard to the value of discarding discolored stem ends of seed tubers, no clear-cut evidence having been presented to show that a decrease in Fusarium wilt can be expected by this method of control.

VASCULAR DISCOLORATION OF STEMS

Very few experimental data have been published on the relation between the vascular discoloration of the stem and the presence of *Fusarium oxysporum*. There are, however, a considerable number of published statements, with little or no experimental data, to the effect that the presence of the vascular discoloration of the stem at the surface of the ground and below is one of the chief signs of Fusarium wilt.

Smith and Swingle (1904 p. 14) state:

> Sections cut at the surface of the ground showed only very few hyphae or none at all. There was, however, a marked browning of the tissues, particularly of the vascular ring.

Manns (1911) in describing the symptoms of Fusarium wilt (p. 306) states:

> Cross sections of the large roots and the stem at a level with the ground or below show some or all of the vessels to be of a brown or dull gray discoloration.

He further states (p. 315):

> No difficulty is experienced in taking the parasitic organisms from the infected tubers and plants.

Bisby (1919) discussing the symptoms of potato wilt in Minnesota (p. 8) says:

> A cross section of the lower stem reveals the browning of the vascular system and often of the other tissues as well. This browning may extend to the tips of the plants, though the bundles are often free from hyphae in these upper discolored areas.

He further says that cultures from wilted plants, particularly from the interior of the stem near the surface of the soil, most frequently yielded *Fusarium oxysporum*. However, he readily obtained cultures of *F. oxysporum* from normal plants killed by frost and showing a browning on the inside of the stems at the surface of the ground. In this case it would appear, as Bisby states, that the browning was due to unfavorable environmental

conditions and that the organism was present more or less saprophytically.

In general it may be said that the use of this sign in diagnosing Fusarium wilt has been based chiefly upon observational evidence, i. e., the presence of a vascular discoloration of the stem accompanying wilt of the plant. It is also based upon the fact that infection obtained by wounding the stem and inserting the inoculum sometimes results in a discoloration of the vascular system by the penetration of the organism. However, as stated by Bisby, browning of the vascular region of the stem may be brought about by frost injury, and the writer will also attempt to prove that browning may also be brought about by other unfavorable environmental conditions.

It is also known that *Fusarium oxysporum* is not the only organism capable of producing this effect. Edson and Shapovalov (1920) found that *F. trichothecioides* Wollenw. was capable of invading the vascular bundles. Link (1916) also obtained in inoculation experiments with *F. trichothecioides* a blackening of the vascular bundles extending even into the petiole and leaf veins. The writer has obtained slight vascular discoloration with such organisms as *Penicillium* sp. and *Rhizopus* sp., and the browning of vascular elements by *Verticillium albo-atrum* is a well-known sign accompanying the wilt caused by that organism. While these organisms may produce symptoms typically different from those of *F. oxysporum*, yet it must be remembered that under field conditions with different environmental factors present we are often concerned with what might be termed atypical cases. Until the differences in symptoms caused by environmental factors are determined, it is not advisable to use such a variable symptom in diagnosing the disease.

It is also true that plants may wilt and die from infection with *Fusarium oxysporum* from the soil, due to a rotting of the roots, without any vascular discoloration of the stem being present. The writer has found this to be true in inoculation experiments as well as under field conditions.

The writer is not aware of any single instance in published reports of this disease where a distinct vascular discoloration of the stem, without accompanying rotting of the parenchyma, has ever been produced experimentally by infection with pure cultures, except where the organism was introduced directly into the vascular elements.

Thus, we may have a browning of these elements not due to *Fusarium oxysporum*, and we may also have a Fusarium wilt produced without any evidence of browning, and under these condi-

tions this sign of the disease would not be of value in determining the amount of infection in the field unless accompanied by other symptoms.

Greenhouse Experiments with Seed Having Vascular Discoloration

EXPERIMENT 1, 1915

Tubers of the Rural New Yorker variety were obtained from a field showing a high percentage of wilt on the college farm at East Lansing, Michigan. The tubers showed a distinct brown discoloration for one-fourth to one-half the length of the tuber, and *Fusarium oxysporum* was isolated from a high percentage of these tubers.

Methods.—Tubers were planted February 9 and dug and examined May 8. All tubers were treated with either mercuric chloride or formaldehyde and planted in sterilized soil. The temperature of the greenhouse was abnormally high for the growth of potatoes, about 85°F. (29.5°C.), and as a result the yellowing and wilting of the vines could not be taken as a positive indication of infection. Diagnosis was made by an examination of the vascular system of the stem and tuber, followed by cultural work.

Results.—SET 1.—Thirteen plants were grown from seed tubers showing vascular discoloration. The results showed 4 healthy plants, 5 with a slight browning of the vascular system, and 4 with a heavy infection of the vascular system from which *Fusarium oxysporum* was isolated.

SET 2.—Nine plants were grown from seed tubers with the discolored portion cut away. The results showed 6 healthy plants, 1 with a slight infection, and 2 with a heavy infection of the vascular system.

SET 3.—Eight infected tubers were cut in half, the bud ends and stem ends being planted separately. The bud-end pieces produced 7 healthy plants and 1 slightly infected. The stem-end pieces produced 2 healthy plants and 6 infected.

SET 4.—Seven tubers were planted showing a slight infection. Four of these produced infected plants from which the causal organism was recovered, while 3 plants remained healthy.

SET 5.—Sixteen tubers were planted showing a slight infection; i. e., discoloration did not extend 1 inch. The stem ends were cut away to the depth of 1 inch and discarded; the seed was then treated with formaldehyde, 10 of the resulting plants showed infection.

Further tests were made in the greenhouse in 1920. Tubers showing vascular discoloration were divided into 2 lots and grown at 17° and 27° C. All plants remained healthy.

These few tests proved that the disease was capable under the conditions of the experiment of living over winter in the tuber and producing an infected plant the following year. However, the typical symptoms of wilt were produced in only a few cases, altho the organism was present in the vascular system of the stems. The bud end of infected tubers usually produced healthy plants, altho an attempt to cut away the stem end even of

slightly infected tubers for a fixed distance of 1 inch did not result in uniformly healthy plants.

One plant of this series showed a condition that the author has frequently found in later experiments and in field trials. The seed tuber showed discoloration of the vascular system and *Fusarium oxysporum* was isolated. The plant produced from this tuber appeared healthy. Upon examination at the close of the experiment the seed-piece was found to be sound, and the causal organism was again isolated from the vascular system. The vascular infection, however, had not progressed far enough to penetrate the tissue of the buds, and the resulting plant remained healthy.

Field Experiments on Seed Transmission

EXPERIMENT 1, 1915

This experiment was conducted on land not previously planted to potatoes for at least 17 years.

Methods.—The infected tubers were all obtained in Michigan and were of the Rural New Yorker variety. They were divided into two groups. 1. Slightly infected seed, i. e., seed in which the vascular discoloration extended not more than ½ inch. 2. Deeply infected seed, i. e., seed with more extensive vascular discoloration. Most of the seed was treated with either mercuric chloride or formaldehyde, either before or after cutting. In other experiments no treatment was given.[1] Notes were taken on the amount of wilt appearing in all rows throughout the experiment, and at digging time each hill was examined for vascular discoloration of the underground stem and also of the tubers produced. In all the following tables the percentages of infected plants are based upon the number of hills germinated, rather than upon the number of hills planted. Healthy tubers of the same variety were used for controls.

Plot 1

Four rows of 65 hills each were planted with healthy seed. Four more rows were planted one-half their length with healthy seed and the other half with slightly infected seed. The seed was cut into stem and bud-end halves, the halves being planted in alternate rows.

Results.—The results of this test are shown in Table 1. The rows planted with infected seed showed considerable wilting and premature dying during August, whereas the healthy rows remained green until frost.

[1] No data are presented in regard to these treatments, as the results did not show any correlation between the various treatments and the amount of resulting disease, and inasmuch as the data are not presented in yields, they were not considered to be a factor in the results presented here.

TABLE 1.—*Percentage of infected plants from healthy seed as compared with plants from seed showing vascular discoloration*

Field Experiment 1. Plot 1

Seed	Plants wilted or with vascular discoloration of stem	Number of blank hills	Resulting number of tubers with vascular discoloration
	Per cent	*Per cent*	*Per cent*
Controls, 4 rows............	4.0	25.4	1.3
Infected seed four ½ rows { Bud ends...	33.8	16.0	12.4
Infected seed four ½ rows { Stem ends..	45.0	50.0	19.3
Controls, four ½ rows......	1.6	41.0	.6

No distinct correlation is found between the number of hills germinated and the vascular discoloration of the seed. The small amount of infected plants present in the control rows was probably due to soil infection but does not materially affect the relation between the healthy and diseased seed. The amount of disease present in the progeny from diseased seed was much higher than from the healthy seed. The amount of disease produced by stem-end pieces was only slightly higher than from bud-end halves. The presence of the very small amount of disease in the control halves of the infected rows does not show any indication of the disease being spread thru the crop by cultivation, as suggested by several authors.

In regard to the vascular discoloration of tubers produced, there were more infected tubers found in the rows planted with infected seed than in the controls. The presence of the causal organism was not determined, but there is certainly a distinct correlation between this vascular discoloration and the presence of vascular discoloration in the mother tuber.

Plot 2

Rows of 65 hills each were planted with deeply infected seed, and these were alternated with rows of healthy seed, the object being not only to compare the two kinds of seed, but also to test the possibility of the disease spreading from row to row.

Results.—Plants from infected seed were badly wilted in August and died prematurely, while the control rows remained

healthy. The results presented in Table 2 again show the much greater number of diseased plants in the rows from seed with vascular discoloration. While the amount of infection was slightly higher in the controls than was found in Plot 1, it is

TABLE 2.—*Comparison of healthy seed with seed having deep vascular discoloration*

Field Experiment 1, Plot 2

Row	Seed	Plants wilted or with vascular discoloration of stem	Number of hills blank	Resulting number of tubers with vascular discoloration
		Per cent	*Per cent*	*Per cent*
1	Healthy.........	4.8	6.0	1.6
2	Diseased.........	68.0	18.1	40.0
3	Healthy.........	4.8	4.6	2.2
4	Diseased.........	64.0	24.3	28.0
5	Healthy.........	8.8	13.6	19.0
6	Healthy.........	10.0	33.0	2.8
7	Diseased.........	75.0	21.1	50.5
8	Healthy.........	9.4	3.3	2.8
9	Healthy.........	8.5	12.1	2.4
10	Healthy.........	3.9	13.6	.8
	Average of healthy and diseased rows			
Rows 7	Healthy.........	7.1	12.3	4.2
3	Diseased.........	69.0	21.1	39.5

not probable that it was spread from adjacent infected rows, inasmuch as Rows 1 and 3 show less infection than other control rows farther removed from the diseased seed. There was no correlation between diseased seed and the number of hills failing to germinate. Altho the averages show greater failure to germi-

nate in the rows planted with diseased seed, this does not hold true when the individual rows are examined. As in Plot 1, there was a greater number of diseased tubers produced from diseased rows than from healthy rows.

Plot 3

This plot was composed of 8 rows of 65 plants each. All seed used in this test was discolored for a considerable depth. The infected portion of some of the seed was cut away to determine the effect upon the transmission of the disease. The bud and stem ends of tubers were kept separate in every case and planted in alternate rows.

Results.—The results presented in Table 3 show that in the case of a deep infection it was not possible to remove successfully all the infected portion of the tuber. There was also no clear relation between the amount of infection present in plants produced from bud ends and stem ends. Neither was there any relation between cutting away the infected portion and the num-

TABLE 3.—*Comparison of plants grown from bud-ends and stem-ends of tubers showing vascular discoloration and also of tubers with this discoloration cut away*

Field Experiment 1, Plot 3

Row	Seed	Plants wilted or with vascular discoloration of stem	Number of blank hills	Number of tubers with vascular discoloration
		Per cent	*Per cent*	*Per cent*
1	No treatment, bud end...	85.0	21.6	37.7
2	No treatment, stem end..	50.0	73.5	22.2
3	No treatment, bud end...	75.0	41.0	45.0
4	No treatment, bud end...	93.0	26.0	47.0
5	No treatment, stem end..	87.0	42.0	35.0
6	Inf. cut away, bud end...	75.0	22.0	32.0
7	Inf. cut away, stem end..	62.0	33.0	19.0
8	Inf. cut away, bud end...	79.0	53.0	36.0

ber of blank hills: there was a slightly greater average percentage (44.4 per cent) of blank hills in the "no treatment" seed than in the seed from which infection was cut away (36 per cent). Also, there was a difference in the number of blank hills planted with stem ends, 56.1 average per cent, as compared with 28.7 average per cent for plants from bud ends. In regard to the resulting tubers showing vascular discoloration, there was no relation either between bud and stem ends or between cut and uncut seed.

Plot 4

In this plot tubers with slight infection were divided into 2 sets, one-half of the tubers having the infected portion cut away. Two rows of 65 plants each were planted in each set.

TABLE 4.—*Effect of cutting away discolored portions of tubers having only a slight discoloration*

Field Experiment 1, Plot 4

Treatment	Plants wilted or with vascular discoloration of stem	Number of blank hills	Number of tubers with vascular discoloration
	Per cent	*Per cent*	*Per cent*
Infection not cut away..	42.0	37.5	17.5
Infection cut away......	28.2	35.0	13.5

Results.—There was less infection from seed showing slight discoloration than was found in Plot 3 with plants from seed with a deep discoloration. It was also noticeable that there were nearly 15 per cent fewer infected plants when the infected portion of the seed was removed. However, the amount of infection was still so high that this was apparently not a very practical method of controlling the disease. There was no apparent difference in the amount of germination. There were slightly fewer resulting tubers showing discoloration when the infected portion of the seed was removed.

EXPERIMENT 2, 1916

Further experiments on the transmission of Fusarium wilt by means of internally infected seed, as well as attempted control measures, were undertaken. The method of work was very similar to that described in the previous experiments except that it was carried out on a larger scale.

The results were so uniformly negative that no detailed data will be presented. Very few of the plants in any of the plots showed even slight symptoms of wilt during the summer. At digging time over 90 per cent of the plants, including not only the infected seed both cut and uncut, and with various treatments, but also all controls, showed a browning of the vascular system of both the stems and tubers. Attempts to isolate the causal organism resulted in failures except in a very few instances out of hundreds of isolations. It is interesting to note that most of the infected seed used in this experiment came from a field showing almost 100 per cent wilt the previous season. The conditions of both soil and air thruout the experiment were radically different from those present the preceding year (Field Experiment 1), when such marked wilting occurred. The detailed meteorological data for this experiment are given on page 17. The weather thruout all of July and part of August was extremely hot and dry in contrast with the moist cool summer of 1915. These conditions, which were found to be unfavorable for infection in the inoculation experiments (p. 17), are also unfavorable for the development of the disease from infected seed.

The type of brown discoloration present in this experiment was not due to infection with Fusarium wilt, but rather to the unfavorable environmental conditions under which the crop was growing. This non-parasitic browning was also found in inoculation experiments (p. 17) and is also mentioned by Edson (1920) as having been observed in plots in the vicinity of Washington.

EXPERIMENT 3

In the spring of 1921 a small lot of Early Ohio tubers showing varying degrees of vascular discoloration were divided up into three lots: slight, medium, and deep discoloration. Each tuber was cut in half and the corresponding halves planted at Lincoln and at Mitchell, Nebraska. Each tuber was divided into stem-end and bud-end halves. The lot at Mitchell was grown under dry conditions. The weather for both locations is shown in Figures 3, 4, and 5.

The fields were examined several times during the growing season and at no time was there any typical wilt present in any of the lots. The results at digging time showed a considerable number of plants with vascular discoloration in the stems and tubers. The number of plants was too small to draw general conclusions, altho there appeared no clear-cut difference in the number of stems showing vascular discoloration under the 2 different

environmental conditions, or in the various lots of seed. Plants from seed with slight discoloration grown at Lincoln had a smaller number of vascular discolored stems and yet these same plants show the greatest number of tubers having vascular discoloration. It was noticeable that the yield was much greater at Mitchell, showing a more favorable environment for the plants.

In Table 5 is seen a comparison of the yield of deeply infected seed with the other 2 lots. While no wilting occurred and the percentage of plants showing vascular discolored stems and tubers was no greater with deeply infected seed than in the other lots, it is clearly seen that the yield per plant was materially lessened both at Mitchell and Lincoln. This shows further evidence that such seed should not be used even tho under certain conditions the disease is not transmitted. The largest yield of healthy tubers was found in the lot planted with slightly infected seed. The yield from bud ends was also considerably greater than the yield from stem ends.

TABLE 5.—*Average yield in grams per plant from slight, medium, and deeply discolored seed tubers*

Field Experiment 3

Seed	Yield per plant in grams	
	Lincoln	Mitchell
Slight discoloration	143	430
Medium discoloration	154	297
Deep discoloration	71	192

CONCLUSIONS

The review of the literature shows that in the earlier papers on Fusarium wilt it was generally accepted that a plant showing Fusarium wilt would produce tubers with vascular discoloration, indicating the presence of the causal organism, and that these tubers would in turn produce an infected plant. Recommended control methods were based upon this hypothesis. The later papers show a decided shift in this attitude and clearly state that this hypothesis does not hold and that the seed tuber is not the chief source of infection; further that the cutting away of sup-

posedly infected portions of the tuber may be really harmful rather than beneficial.

The experimental evidence presented in this paper shows that in the greenhouse, in tests where sterilized soil was used, the disease was carried over in the tuber. The percentage of infection from such tubers was small, but still as high as in the previous experiments where artificial inoculations with pure cultures were employed. Very often infected tubers showing vascular discoloration, and also yielding the causal organism in pure culture, failed to produce wilted plants. In many instances, altho no wilting was produced, the causal organism was found to be present in the stem and roots of the plants. Experiments conducted in the field showed that under the conditions of Field Experiment 1 (p. 29) Fusarium wilt was transmitted thru the seed tubers and that the amount of resulting disease was directly proportional to the severity of the infection in the seed, as indicated by the depth of discoloration of the vascular system. It was also found that in cases of slight discoloration attempts to remove the infected portion of the tuber resulted in a decrease of about 14 per cent in the amount of disease. With deeply infected tubers, such attempted removal of infected portions of the seed resulted in no decrease in the amount of disease, and in these deeply infected tubers there was no appreciable difference in the amount of disease in the progeny of stem and bud-end seed-pieces.

It was also shown the following year (Field Experiment 2, p. 33) that under certain other environmental conditions the disease was not transmitted thru the tubers and that vascular discoloration of the stem does not always give a good indication of the amount of disease present, altho, as indicated in Experiment 3 (p. 34), the depth of the vascular discoloration can often be correlated with a decrease in yield, thus indicating a lowering of the vitality of the seed.

Vascular discoloration of both stem and tuber was often found in the absence of any causal organism. This was particularly marked in Experiment 2, (p. 33) and Inoculation Experiment 1, (p. 17) this appearing to be due directly to the hot, dry weather. The same type of discoloration was observed in 1921 on potatoes grown in western Nebraska under dry land conditions. Hot weather prevailed thruout the summer and there was very little moisture present until late in the season. The crop, however, appeared very thrifty and healthy in the fields and the potatoes were certified for seed. The sale of these potatoes outside of the State resulted in many complaints, claiming that

the seed was poor in general vigor and was infected with Fusarium wilt. This was due partly to the high amount of vascular discoloration in the seed, altho there was no wilt present in the field. The tubers were rather badly malformed owing to renewed growth late in the season. Nevertheless, this stock when planted at various points in the South showed the smallest amount of disease and the greatest yields of any of the Nebraska potatoes tested in the same trials.

Thus, it is evident that vascular discoloration is not a good indication of Fusarium wilt, altho under certain environmental conditions we do get a considerable amount of wilt by planting such seed, while under other conditions no wilting results. It is to be remembered that in the plots where the disease resulted from planting seed having vascular discoloration the tubers were obtained from stock known to be infected the previous year as indicated by the large amount of wilt, and not simply tubers showing vascular discoloration. In other words, vascular discoloration is not a good criterion of the presence of Fusarium unless it be produced by a wilted plant, and even then the chances are that it will not reproduce the disease the following year by means of seed infection.

INFECTION FROM THE SOIL THRU THE ROOTS OR STEM

It is a well-known fact that *Fusarium oxysporum* can live as a soil saprophyte for many years. It has also been claimed by various workers that *F. oxysporum* causes a wilt of the potato plant by the entrance of the organism thru the root hairs from the soil. While this method of infection has been generally accepted as one of the modes of entrance of the organism, very little positive experimental work has been published to prove this point. The following work was undertaken in an attempt to either prove or disprove this theory, and if true, to determine the conditions favoring such a method of infection.

The author has observed many cases in various parts of the United States, but especially in Michigan and Nebraska, where healthy seed planted on land not previously cropped has resulted in a considerable number of wilted plants. These plants appeared to have become infected thru the roots; very often there was only a discoloration of the vascular system without any root rotting and without a rot of the seed-piece. It was this type of infection that prevailed in Michigan in 1914 and 1915 and lead to the undertaking of the present investigation.

Review of Literature

Smith and Swingle (1904) in their early description of the disease state (p. 13):

> The disease ordinarily enters the plant through the roots and slowly spreads until the whole root system, a few centimeters of the lower part of the stem, the underground stems bearing the tubers, and the tubers themselves are invaded by the fungus.

They present no detailed experimental evidence to support their observations.

Manns (1911) also claims that the disease makes its attack thru the root system. This results in the root hairs and smaller secondary roots of affected plants being entirely destroyed and thus causing the premature death of the plant. He found by planting healthy seed on heavily infested soil as compared with slightly infested soil that there was more wilt and a reduction in yield from 125.6 bushels to 90 bushels per acre. He tested the infection powers of "sick" soil by adding a little of this soil to hills of potatoes growing in sterilized soil. He says (p. 317):

> The disease came on much more definitely under the sick soil infection than it did where pure artificial cultures were used without incisions or root injury. The great difference between sick soil infection. and that from pure cultures or even internal seed infection, is that in the use of sick soil the roots are attacked at practically every point, while with pure cultures, or seed internally infected, the fungus attacks only in close proximity to the main root, while most of the secondary roots and the root hairs remain healthy.

He does not, however, give any detailed data in regard to infection from the soil by use of pure cultures.

Link (1916) does not report uniformly successful results with soil inoculations of *Fusarium oxysporum*. He reports one experiment of 5 plants in which the soil was inoculated with rice cultures of *F. oxysporum*. No plants came up in the inoculated soil, whereas the controls appeared healthy. He states that this experiment was repeated several times, but in no case were the results so striking. In fact, in one experiment no infection resulted at all. He stated that plants grown in soil inoculated with *F. oxysporum* showed severe lesions of roots and stolons. He believed that this root injury is just as important in producing wilt symptoms as vascular mycoses. As to the lack of uniformity in the results, he says (p. 188):

> The soil in these experiments surely was more severely infected with the organisms than soil under field conditions can be, yet there were many plants grown in such soil that showed no infection whatsoever. Less success in producing wilt was observed as the season progressed. It remains a question whether this is due to a loss in vitality or to a

gain in resistance in the plants, due to a change in the soil, tubers, or the organism.

Pratt (1916) studied the amount of disease present when healthy seed was planted on virgin desert soil in Idaho. He found wilt to be present in every plot, and isolated the organism from stems showing vascular discoloration. He also states that 80 per cent of the tubers showing vascular discoloration yielded cultures of either *Fusarium oxysporum* or *F. radicicola.* Basing his percentages on this basis, he found on tubers grown on land previously planted to alfalfa and grain 26 per cent vascular infection, while on the virgin desert land plots he found 29.3 per cent.

Bisby (1919), in discussing the results of attempted infection of potato plants by artificially infected soil or seed-piece, says they were often unsuccessful and concludes (p. 12):

> It is evident that *F. oxysporum* is not vigorously parasitic to actively growing potato plants.

He thought that these results may have been due to too low a temperature and made further tests with heavily inoculated soil at soil temperatures of 20° to 30° C. with a high humidity, and produced a stem rot or "foot rot" of the plants. He further states that the higher temperatures in the warm chambers were unfavorable to the potato and the plants died sooner than corresponding plants left outside.

MacMillan (1919) states that in only 6 plants out of many hundreds examined in 1916 and 1917 was root infection determined as the probable cause of Fusarium wilt in the Greeley section of Colorado. He notes that in 1918, when the soil temperature was 6°F. above the average for the month of June of the two preceding years, plants of the Charles Downing variety were badly diseased by being attacked thru the fine roots and root hairs and concludes (p. 284):

> Higher temperatures seem to be necessary for root infection.

Haskell (1919) finds that Fusarium wilt in Dutchess County, New York, is primarily a root and stem rot. He was the first worker to report detailed experimental work under controlled temperatures. In 1915 he inoculated sterilized soil with pure cultures of *Fusarium oxysporum*, one plot before planting, another when the plants were 6 inches high, and the third plot left as a check; the number of plants is not stated. The results were not uniform and this he attributed to the fact that the greenhouse temperatures were too low for infection, 18°-21° in the day and

13°-15.5 C. at night. He repeated this, using the same soil and growing the plants in the summer when the temperatures were higher, and found that many of the new tubers showed marked stem-end browning, from which the fungus was isolated. Another experiment was conducted with 6 plants, 4 inoculated with a flask culture of *F. oxysporum* No. 3395 (the same as Strain No. 8, used by the writer in many experiments). The plants were kept in a water bath at 36° (air temperature not given). He found that the root systems became diseased and that some of the stems showed lesions, and the organism was isolated. He states (p. 237):

> Although the tops of the diseased plants did not resemble those that are affected with Fusarium wilt as it appears in the field, the affected root system and the lesions on the outside and within the stem were exactly like those that occur in the field.

He repeated this experiment, keeping the crocks in the water bath at 36°-39°C. Again he was apparently unable to produce typical Fusarium wilted plants, altho he states (p. 237):

> An examination of the xylem of inoculated plants showed it to be badly discolored in most of the stems. In some instances the checks also showed this condition but not so badly.

The writer is in doubt as to just how much of this discoloration of the xylem reported by Haskell was due to the extremely high temperatures, as vascular browning can be produced by submitting plants to very high temperatures even for short periods, and this diseased tissue becomes easily infected with Fusaria from the soil. It is clear, however, from Haskell's work that even with high soil temperatures it is difficult to obtain 100 per cent infection or to produce typical field symptoms of wilt. Haskell also tested soil infection by growing healthy seed in the greenhouse in soil brought from a badly diseased field. Twelve plants were grown in this soil and 12 in sterilized soil. No typical symptoms of Fusarium wilt appeared. The roots in both plots appeared diseased altho *Fusarium oxysporum* was isolated only from the plants on unsterilized soil. In regard to soil infection, Haskell further makes the statement (p. 242):

> Land that has produced a diseased crop one year is practically certain to do so the next if potatoes are planted.

The writer is at a loss to understand this statement in the light of the experiments reported, showing infection to depend upon an extremely high soil temperature.

As regards the influence of climatic factors on the disease as observed in the field, Haskell states that hot weather is favor-

able for the disease and unfavorable for the host and that it is during the hot weather that wilt develops. How then account for the writer's results in Michigan in 1916 when practically no wilting appeared on badly infested soil during the hottest summer in years. Haskell concludes (p. 252) that—

> temperature is probably the most important factor limiting the development of Fusarium wilt in southeastern New York.

He believes that the amount of rainfall and hence soil moisture has very little to do with the prevalence of the disease. He does think, however, that with a low soil moisture diseased plants will wilt quicker.

This review of the literature shows a general conformity of opinion among the earlier workers on this disease, who claim that infection can take place from the soil thru the roots. There are some conflicting statements as to whether this type of infection is confined to the vascular system of the roots and stems, or whether it results in a rotting of the root system. It is noticeable that the only writer working under western irrigated conditions, MacMillan (1919), claims that only a very small amount of infection occurs thru the roots. It is generally stated that high soil temperatures favor the development of this type of infection. Haskell reports successful soil inoculation experiments at soil temperatures of 36° to 40°C. This temperature is the extreme maximum temperature for the growth of both host and organism. No experimental work is reported regarding the relation of soil moisture to infection.

Field Experiments with Infested Soil, 1914-15-16

During 1914 a field of Rural New Yorker potatoes on the college farm at the Michigan Agricultural College showed over 90 per cent of the plants affected with Fusarium wilt. The plants wilted early in the year. *Fusarium oxysporum* was repeatedly isolated from stems and tubers of wilted plants showing vascular discoloration as well as from the soil.

In 1915 four rows, 330 feet long, of this heavily infested soil were planted with healthy seed. Very few of the plants showed the early symptoms of the disease and very little wilting occurred until just before digging time. The plants in these 4 rows were found to be 47 per cent infected as determined by actual wilting, vascular discoloration, root infection, and cultural examinations; while the same seed planted on soil not previously planted to potatoes showed only 3 per cent infection.

In 1916 four more rows were planted on this land that had

been in clover the previous year. A few plants were wilted in August and at the end of the experiment only about 20 plants, or 4.5 per cent of the total number of plants, showed wilting symptoms. When dug, the stems and tubers of all plants showed vascular browning similar to that noted on page 34, but no successful isolations were made except from the 20 wilted plants. The weather conditions for this year are given on page 17. An examination of the infected plants showed in every instance that infection took place thru the roots rather than thru the seed-piece. The amount of rotting of the root system was very slight, and no rotting of the underground stems was observed except in the few cases where wilting took place early in the season and the plants were not dug until the end of the experiment. The infection in practically all cases was confined to the small root hairs and the vascular system of the main roots and underground stem.

While this experiment shows at first glance a reduction of the wilt, the first year from 90 per cent to 47 per cent, and after one year's rotation to 3 per cent, it must be remembered that climatic conditions were probably a greater factor than was the rotation used.

Greenhouse Experiments

In the following experiments, only vigorous plants grown from healthy seed were used, the seed tubers being carefully examined for the absence of vascular infection before planting. The soil inoculations were made by mixing thoroly with the soil a suspension of spores and mycelium in sterile water.

EXPERIMENT 1, 1915

Tubers of the Green Mountain variety were planted February 8. The inoculations were made when the plants were about one foot in height on March 1, and the final results were taken May 10. The inoculum was obtained from cultures of *Fusarium oxysporum* No. 1.

Set 1.—Nine plants were grown in inoculated soil. None of these plants showed typical wilt and at the close of the experiment no browning of the vascular system of either the roots or the stems was observed.

Set 2.—Ten plants were grown in inoculated soil. The roots of these plants were slightly injured, but not enough to impair their vigor. Three of these plants showed a very heavy infection, the typical Fusarium wilt appeared rather early, and the fungus was found to be present very abundantly in the vascular system. The organism was isolated in pure culture. Four plants showed only a slight infection, the wilt not being serious enough to kill the plants. Three plants remained healthy thruout the experiment.

Set 3.—Five plants were grown in soil previously inoculated with *Rhizoctonia solani* Kuhn, and later with *Fusarium oxysporum*. This was

done in order to observe the possibility of *F. oxysporum* obtaining an entrance into the roots thru the Rhizoctonia lesions. No wilting occurred and no browning of the vascular system was observed. Rhizoctonia lesions were found on 2 of these plants.

Set 4.—Five plants were grown in soil inoculated by placing pieces of potatoes internally infected with *Fusarium oxysporum* in the soil close to the roots. Three of these plants showed a slight wilt and a slight browning of the vascular system. Two plants remained healthy.

Set 5.—Five plants were grown in soil inoculated as in Set 4, but the infected pieces were placed next to injured roots. Two plants showed a heavy infection of the wilt similar to those of Set 2. Three plants remained healthy.

Set 6.—Twelve plants were used as controls. In some a wilting occurred somewhat similar to that of the inoculated plants, so the wilting of the plants could not be taken as a sure sign of the disease. However, no browning of the vascular system was found in any of the controls and careful microscopic and cultural work failed to reveal the presence of the fungus.

EXPERIMENT 2, NOVEMBER, 1915

Potato plants of the Russet Rural variety were used in the greenhouse for inoculation experiments similar to the previous ones. The inoculum was obtained from cultures of *Fusarium oxysporum* No. 1.

Set 1.—The roots of 2 plants were injured and the soil inoculated. One of these plants produced typical wilt and showed vascular browning. The other plant remained healthy.

Set 2.—Two plants were inoculated as in Set 1 but the roots were not inujred. Both of these plants remained healthy.

Set 3.—Four plants used as controls remained healthy.

EXPERIMENT 3, MARCH, 1917

Fourteen tubers of the Rural New Yorker variety were planted in the greenhouse. The soil was inoculated with *Fusarium oxysporum* No. 8 just as the shoots were showing at the surface of the ground. One plant showed slight symptoms of the wilt, and the fine rootlets when observed under the microscope were found to be invaded by the fungus. The other 13 plants remained healthy. Three plants used as controls remained healthy.

EXPERIMENT 4, MARCH, 1917

Four tubers of the Rural New Yorker variety were planted in the soil previously used in Experiment 3. All the plants when very young showed symptoms of the wilt. When these plants were dug up and examined, the roots were found to be heavily invaded and the entire underground portion of the plant showed the presence of the fungus. Isolations were made from each plant and the organism recovered in all cases. Four other plants kept as controls remained healthy.

EXPERIMENT 5, MARCH, 1917

Twelve plants of the same variety as those of the previous experiment were used. The soil was inoculated just as the sprouts were breaking thru the ground. All the plants remained healthy.

EXPERIMENT 6, 1920

In these tests the soil was sterilized and then inoculated with 3-weeks-old rice cultures of *Fusarium oxysporum* No. 33. The experiment was started February 21; the results were taken May 5. Two temperatures were used.

Set 1.—Five plants were placed in a greenhouse having a mean temperature of about 17° C. All 5 plants remained healthy.

Set 2.—Three plants were grown in a greenhouse with a mean temperature of about 27° C. Plant 1 showed no wilt, 2 stems out of 4 had brown lesions at the base of the stem, and there was slight root injury but no browning of the vascular system. *Fusarium oxysporum* was recovered from the lesions. All the underground stems of plant 2 showed lesions, also some of the roots and stolons. There was no wilt or vascular infection. *F. oxysporum* was recovered from the lesions. Plant 3 showed no wilt or vascular invasion, but 2 of the stems showed numerous lesions and considerable root injury. *F. oxysporum* was recovered from the stems and the roots. In these plants the lesions caused by *F. oxysporum* on the underground stems were entirely superficial, while on the roots the penetration was deeper and the injury much greater, often cutting off the root.

EXPERIMENT 7, 1920

On February 16, 6 pots, 4 with inoculated soil and 2 uninoculated controls were planted with Irish Cobbler seed and kept at 25° C. until March 10 when they were all about 6 inches high with a good even growth and no signs of disease. They were then removed to a greenhouse kept at 30°-33°. On March 20 the plants growing in inoculated soil were badly yellowed and wilted. The controls, altho entirely dead above ground, showed no signs of disease in the underground parts, the stem, roots, and stolons being healthy. *Fusarium oxysporum* was recovered from the rotted portion of the stem of inoculated plants.

A summary of these soil inoculation tests shows that out of 80 plants inoculated in various ways only 14 wilted, 11 were infected but did not wilt, and 55 remained healthy. The results show that the greatest amount of wilt was obtained when the soil was inoculated before planting or when the plant was very young. There was no difference in the amount of infection in the different varieties of potatoes. Injury to the roots resulted in a higher percentage of infection. The results also indicate that soil temperature and possibly air temperature has a considerable influence upon the infection and progress of the disease.

Relation of Soil Temperature to Infection of Roots from the Soil

Further experiments under controlled conditions were started to determine the relation of soil temperature and moisture to the disease.

Methods.—In these experiments, "Wisconsin soil temperature tanks," previously described by Jones (1917), were used to control the soil temperatures. In the experiments at Nebraska slight modifications in these

"tanks" were made, but the principle employed was essentially the same. In these "tanks" the plants were grown in galvanized iron cans immersed in a well-insulated water bath, 21x39x27½ inches in size, heated electrically and controlled by electric thermostats. The temperature of the soil in all the following "tank" experiments was taken twice daily at the depth of 3 inches. The soil moisture content was kept approximately constant by weighing the pots and adding the necessary water about 3 times a week, and as the plants grew larger, the water was added daily. In some of the experiments the surface of the soil was covered with mineral wool and in the latter experiments with crushed stone to prevent a rapid change in the temperature of the surface soil and also to prevent the drying out of the surface soil. Water was added thru a test tube to an inverted clay pot in the center of the container and was also added to the surface of the soil. With this combination of surface and subsoil watering the moisture was found after numerous tests to be very well distributed throut the soil. It is obvious of course that the soil moisture did not remain constant during the experiment, and varied according to the rapidity of transpirtation and the frequency of watering. The soil was only at the specified moisture content immediately after watering and gradually decreased until the next watering. It is also true that during the experiment the soil would settle, and this packing would result in a decrease in the moisture holding capacity. This is counteracted to some extent by the increase in the weight of the plant, this increase not being taken into account in the weighings. The following percentages show an average of several tests made to determine the changes during the experiment in the moisture content of the soil with growing potato plants. Composite samples were taken from various parts of the container.

	Set 1 Per cent	Set 2 Per cent	Set 3 Per cent
Moisture content (dry weight) at start......	13.6	20.4	27.2
Moisture content (dry weight) at end......	14.0	20.6	23.8

Thus we find the greatest change occurring at the higher moisture contents where the weight of the plant is greater than at the lower soil moistures: the cans at 80 per cent of the moisture holding capacity[1] (27.2 per cent dry weight) being 70 per cent at the close of the experiment. The difference at 40 and 60 per cent of the moisture holding capacity was very slight.

The plants were watered often enough so that it was never necessary to add more than 200 c. c. of water, and usually only about 100 c. c., so that just previous to watering or when the soil was at the lowest moisture content it was usually not more than 10 per cent of the moisture holding capacity below the desired point. In the late spring or with high air temperature when the transpiration was rapid, the difference was slightly greater than the above. However, as all cans in the experiment were handled in the same way, the differences are relative and do not interfere with the interpretations placed upon the results.

[1] The moisture holding capacity was determined with the 25 cm. tubes, which were filled with air dry soil and tapped 10 times from a height of 3 inches, placed in water for 6 to 8 hours, and allowed to drain 3 to 4 hours. This determination was often checked with the 1 cm. cups, in which filter paper was inserted over the screen, the cups then filled with air-dry soil, tapped on the side 5 times, smoothed off, placed in water for 1 hour, and drained 15 minutes. In all the following experiments the soil moisture is expressed as per cent of the moisture holding capacity rather than as per cent dry weight.

EXPERIMENT 1, FEBRUARY 16 TO APRIL 8, 1920

Methods.—Healthy Irish Cobbler potatoes were treated with formaldehyde and sprouted for 2 weeks in a cool place. The tubers were cut in half for seed. The soil used was one part leaf mold and 2½ parts soil sterilized for 8 hours at 2½ pounds pressure. About 3,000 grams of soil, dry weight, was used in each can. The soil had a moisture holding capacity of 40.5 per cent dry weight. During the experiment it was kept at 29.0 per cent dry weight, or about 72 per cent of the moisture holding capacity. The soil was inoculated just previous to planting with a spore suspension in water of a 14-day-old rice culture of *Fusarium oxysporum* No. 33. Immediately after planting the cans were removed to the desired temperature.

Five different soil temperatures were used; 9 plants being grown at each temperature, 6 with inoculatd soil, and 3 uninoculated controls. The temperatures used were 12°, 18°, 24°, 30°, and 36° C. Readings were taken twice daily and the average thruout the experiment was 11.8°, 17.5°, 23.8°, 30.1°, and 35.7°. The temperatures did not vary more than 1° on each side of the average. The air temperature was maintained at approximately 17°.

Results.—The potatoes were planted February 16, and by February 23 the sprouts were coming thru at 18°, 24°, and 30°C. The most rapid growth was at 24°. At 12°, the plants were slower in getting started. They were all up on March 3. At 36° all the seed-pieces rotted before the sprouts started. The plants at 18° produced a very even stand of vigorous, healthy plants, and these plants along with those at 12° remained healthy thruout the experiment with no apparent difference between the inoculated plants and the controls. One plant at 30° was wilted down by March 10 and the other plants at this temperature all showed a yellowing of the lower leaves apparently due to the high soil temperature.

The plants were dug on April 8 and the tops, underground stems, and roots examined and cultures made from plants showing disease. The results of this experiment are tabulated in Table 6. It is apparent from this table that no infection took place at 12° and 18° C. At 24° there was no marked difference in the tops of control and inoculated plants; in only one case were the tops wilted. Three plants had slight root injury and *Fusarium oxysporum* was isolated. At 30° 5 of the 6 inoculated plants showed varying but distinct symptoms of wilt and when examined were found to have vascular discoloration of the stem and in 4 instances the underground stem was rotted. *F. oxysporum* was isolated from all roots showing injury and from stems showing either vascular discoloration or rotting.

It is evident from this experiment that temperatures of 18° and below are unfavorable for the development of the disease

TABLE 6.—*Relation of soil temperature to infection of Irish Cobbler potatoes from soil inoculated with Fusarium oxysporum No. 33.*

Experiment 1[1]

Soil temperature	Treatment of plants		Symptoms				Cultures of *F. oxysporum*	
			Wilt	Vascular discoloration	Stem rot	Root injury	From roots	From stems
Degrees C. 12	6 inoculated plants, 3 controls, all healthy							
18	6 inoculated plants, 3 controls, all healthy							
24	Inoculated	1	—	—	—	1	+	—
		2	—	—	—	1	+	—
		3	2	2	3	1	—	+
		4	—	—	—	—	0	0
		5	—	—	—	—	—	—
		6	—	1	—	—	—	—
	Control...	7	—	—	—	—	0	0
		8	—	—	—	—	0	—
		9	—	—	—	—	—	—
30	Inoculated	1	3	3	3	3	+	+
		2	2	2	2	1	+	+
		3	—	—	—	1	+	—
		4	3	3	3	3	+	+
		5	1	—	—	1	+	—
		6	3	3	3	—	—	—
	Control...	7	—	—	—	—	—	—
		8	—	—	—	1	0	0
		9	1	—	1	—	—	—
36	6 inoculated plants, 3 controls, all seed rotted							

The figures in Tables 8 to 12 represent the degree of infection; 1 = slight, 2 = medium, 3 = serious. In the last two columns the results of isolations are shown; 0 = negative culture, + = positive culture, — = no culture made.

[1] The moisture content of the soil was kept as 72 per cent of the moisture holding capacity, or 29 per cent dry weight.

and that above 18° the amount of infection in the inoculated plants increased with the increase in temperature up to 30°C. At 36° the temperature was too high for good germination of the seed. It is noticeable that only 6 plants in the experiment

showed positive symptoms of wilt or stem injury, whereas 8 plants showed root injury. The controls remained healthy except at 36° and in the case of one stem of a control plant at 30°.

EXPERIMENT 2, APRIL, 1920

The methods used in this experiment were similar to those in the previous experiment except that Rural New Yorker potatoes were used instead of Irish Cobblers. The soil moisture content was also lowered. The moisture holding content of the soil used was 49.7 per cent dry weight. The sets at the various temperatures were divided into two series, one in which the soil was held at 51 per cent of the moisture holding capacity (25.3 per cent dry weight) and the other at 57 per cent (28.3 per cent dry weight). The following temperatures were used.

Set No. 1: Air temperature 17°C. Soil temperature 18°, 22°, 26°, 30°, and 34°C.

Set No. 2: Air temperature 27°C. Soil temperature 18° and 27°C.

The seed-pieces at all the temperatures were completely rotted before the sprouts got above the ground, both in the controls and in the inoculated pots. There were a few weak sprouts appearing above the ground but not enough to continue the experiment.

EXPERIMENT 3, NOVEMBER 5 TO FEBRUARY 4, 1921

This experiment and the following soil temperature experiments were conducted at the Nebraska Agricultural Experiment Station; and while the same general methods as those outlined in Experiment 1 were used, there were slight differences in each experiment. In Experiment 3 an attempt was made to obtain further information in regard to the effect of soil moisture as well as temperature upon the disease; 2 different soil moistures were therefore used at each temperature.

Methods.—Owing to the fact that no infection was obtained in Experiment 1 at the low soil temperature of 12°, this temperature was omitted in the present experiment and the soil held at 4° intervals instead of the 6° C. intervals as in Experiment 1. Six "Wisconsin soil temperature tanks" were used. In each "tank" 8 cans holding about 10 kilos of soil (dry weight) were used. The same amount of soil was used in each can. The soil in 4 of these cans at each temperature was kept at approximately 40 per cent of the moisture holding capacity and in the other 4 at 60 per cent of the moisture holding capacity. The moisture holding capacity of the soil was determined to be 38.2 per cent dry weight with the 25 cm. cup method previously described. Six soil temperatures were used, 16°, 20°, 24°, 28°, 32°, and 36°. The averages of the readings taken twice daily at a depth of 3 inches were 17°, 20.3°, 23.5°, 27.6°, 31.8°, and 35.5°. These temperatures varied slightly thru-

out the experiment but the extremes did not overlap and usually the temperatures did not vary more than 1°. The air temperature was kept at approximately 18°, altho during the last 2 weeks of the experiment the air temperature ran slightly higher, averaging about 20°. The soil used was disinfected with formaldehyde and allowed to air out before planting. All the soil was inoculated just before planting with 14-day-old rice cultures of *Fusarium oxysporum* No. 33. Early Ohio tubers were then cut and sprouted in damp excelsior and when planted the sprouts were ¼ to ½ inch long. Immediately after planting, the cans were placed at the desired temperature and brought up to the desired soil moisture content, the water being added as in Experiment 1.

Results.—The plants were examined daily thruout the experiment and any symptoms of disease were noted. These along with the results obtained at digging time are presented briefly in Table 7. The only temperature at which positive wilting symptoms appeared was 36°C. At this temperature many of the sprouts appeared to be killed before they reached the surface of the ground. They then sent out secondary sprouts which produced tops never exceeding 10 inches in height. Both control plants remained healthy. At 32° no wilting occurred, altho most of the plants were considerably dwarfed as at 36°. It was noticeable at these 2 high temperatures, and especially at 36°, that the root systems were very small and remained near the surface of the soil. This combined with the high soil temperature made it impossible to keep the upper 2 inches of soil at the same moisture content as the rest of the pots, even when insulated with mineral wool. Thus the actual soil moisture surrounding the roots at 36° was considerably less than indicated in the table.

No vascular discoloration of the stems was found in any of the control plants at any of the temperatures. There were no marked differences in the amounts of vascular discoloration at any of the temperatures between 20° and 36°C. As regards soil moisture, 10 plants at 60 per cent of the moisture holding capacity showed vascular discoloration and only 3 at 40 per cent. The amount of stem rot was found to run parallel with the vascular discoloration and in most cases, the 2 symptoms appeared on the same plant. The amount of root injury appeared to be greatest at temperatures from 24° to 36°, altho there was very slight injury to 2 plants at 16°. At the high temperatures the injury appeared greater and isolations from these roots always yielded *Fusarium oxysporum*. Three control plants showed root injury at 28° and 32°, and *F. oxysporum* was recovered from one of them. The causal organism was also recovered from the stems of 8 inoculated plants showing either vascular discoloration or stem rot, while 2 plants with similar symptoms gave negative results in culture.

TABLE 7.—*Relation of soil temperature and moisture to infection of Early Ohio potatoes from soil inoculated with Fusarium oxysporum No. 33*

Experiment 3

Soil temperature	Soil moisture[1]	Treatment of plants		Symptoms: Wilt	Symptoms: Vascular discoloration	Symptoms: Stem rot	Symptoms: Root injury	Cultures of *F. oxysporum*: From roots	Cultures of *F. oxysporum*: From stems
Degrees C.	*Per cent*								
16	40	Control	1	—	—	—	—	—	—
		Inoculated	2	—	—	1	1	—	—
			3	—	—	—	—	—	—
			4	—	—	—	1	—	—
	60	Control	1	—	—	—	—	—	—
		Inoculated	2	—	—	—	—	—	—
			3	—	1	1	—	—	+
			4	—	—	—	—	—	—
20	40	Control	1	—	—	—	—	—	—
		Inoculated	2	—	—	—	—	—	—
			3	—	1	1	—	—	—
			4	—	—	—	—	—	—
	60	Control	1	—	—	—	—	—	—
		Inoculated	2	—	—	—	—	—	—
			3	—	1	1	—	—	+
			4	—	1	1	—	—	+
24	40	Control	1	—	—	—	—	—	—
		Inoculated	2	—	—	—	—	—	—
			3	—	—	—	1	—	—
			4	—	—	—	1	—	—
	60	Control	1	—	—	—	—	—	—
		Inoculated	2	—	1	1	—	—	+
			3	—	1	1	—	—	0
			4	—	1	—	1	—	—
28	40	Control	1	—	—	1	1	+	—
		Inoculated	2	—	—	—	—	—	—
			3	—	3	2	3	+	+
			4	—	—	—	—	—	—
	60	Control	1	—	—	—	1	—	—
		Inoculated	2	—	—	—	1	—	—
			3	—	—	—	1	—	—
			4	—	1	1	1	—	+
32	40	Control	1	—	—	—	1	0	—
		Inoculated	2	—	—	—	2	+	—
			3	no	germination				
			4	—	—	1	2	+	—
	60	Control	1	—	—	—	—	—	—
		Inoculated	2	—	1	1	1	—	+
			3	—	—	—	1	+	
			4	—	—	—	1	—	
36	40	Control	1	—	—	—	—	—	
		Inoculated	2	no	germination				
			3	no	germination				
			4	1	1	1	1	[illegible]	+
	60	Control	1	—	—	—	—	—	—
		Inoculated	2	—	—	—	1	+	
			3	1	1	1	3	+	
			4	3	3	—	3	0	0

[1] Soil moistures are expressed in per cent of the moisture holding capacity (38 per cent dry weight).

The results of this experiment are not as clear-cut and conclusive as those of Experiment 1. The per cent of infection was not as high; and while 30° was found to be the optimum soil temperature for the disease in Experiment 1, no positive wilting occurred at either 28° or 32° C. in this experiment. It is to be remembered that Early Ohio seed was used in this experiment while Irish Cobbler seed was used in Experiment 1. The results indicated that low soil moistures thruout the experiment were not favorable for the production of the wilt. This experiment was the first one in which plants were grown successfully at 36°, and while the plants grew and the amount of infection was greatest at this temperature, the appearance of the plants, especially of the root system, was very abnormal and neither the type of plant growth nor the conditions under which they were grown are comparable with natural field conditions.

EXPERIMENT 4, MARCH 3 TO MAY 12, 1921

This experiment differed from Experiment 3 in that higher soil moistures were used and no plants were kept at a constant temperature of 36°C. In the previous experiments the percentage of germination at this high temperature was very low and when germination took place the resulting plants were usually very abnormal and the unnatural growth increased the difficulty of recognizing the symptoms of the disease. In order to obviate this and also to study the effect of changing soil temperatures, the plants growing at 18° and 36° were alternated.

Methods.—Two different soil moistures were used. The moisture holding capacity of the soil determined by the 25 cm. cup method was 35 per cent dry weight. One-half of the cans were held at 60 per cent of the moisture holding capacity (21 per cent dry weight), which was the same as the highest moisture content in Experiment 3. The other half were held at 80 per cent of the moisture holding capacity (28 per cent dry weight). In order to prevent the drying out of the upper 2 inches of soil, the moisture content was kept constant by both subsoil and surface watering for several days previous to planting. Immediately after planting, the soil in each container was covered with 900 grams of crushed rock.[1] The same soil temperatures were used in Experiment 3 except at 36°C. One set of 8 plants was started at 18° soil temperature, and when the plants were about 3 to 6 inches tall at the end of 25 days and apparently healthy, they were changed to a soil temperature of 36° and remained at this temperature during the rest of the experiment. In the same way, one set of 8 plants was started at 36° and at the end of 25 days none of these plants were above ground. They were then removed to a temperature of 18° for the rest of the experiment. The soil was prepared

[1] This was found to be a more satisfactory means of decreasing evaporation from the surface soil and keeping the upper layer of soil at a constant temperature than the mineral wool used in the previous experiments. It was also much easier to handle and allowed of some surface watering.

in the same way as for Experiment 3 and tubers were used from the same source and treated in the same way. Just previous to planting, the soil was inoculated with cultures of *Fusarium oxysporum* No. 35 grown on rice. The containers were placed at the desired temperatures immediately after planting. The soil temperatures were taken at a depth of 3 inches 3 times daily and did not vary more than 1° from the desired temperature except in one set.[1] The air temperature was kept at approximately 18° except during the last few weeks of the experiment, when it sometimes rose to 25° during the middle of the day.

Results.—The plants started at 18° and changed to 36° C. were so badly wilted that they were dug and examined on May 2; the remainder of the plants were all dug on May 12. The results of the experiment are tabulated in Table 8. It was noticeable that at all the temperatures used many of the plants showed a yellowing and wilting of the lower leaves at various times during the experiment and later recovered and when examined at digging time were found to be healthy. Such plants are not listed in the table as diseased. The quick change of soil temperature with the plants started at 18° and changed to 36° resulted in a rapid wilting and drying of the lower half of the plant. After about one week, however, these leaves dropped off, the plants, apparently becoming adapted to the high temperature, appeared perfectly healthy, and later the disease appeared and wilting became complete in some cases.

At 18°C. no definite wilting was observed except in the case of one control that wilted from other causes. No wilt occurred at 24° except in one control plant. Four plants, 2 at each soil moisture, showed either slight stem rot or slight root injury. At 28° one plant wilted and one other plant showed definite signs of the disease in the underground portion. At 32° the results do not agree with the previous experiments; practically no infection was found, only 2 of the plants showing a slight rotting at the base of the stem. The plants started at 18° and changed to 36° showed a marked difference from the plants at the other temperatures. All of these, including the controls, showed wilt symptoms. The vascular system of the stem of both controls was slightly discolored but no root infection or stem rot was present and cultures from the stem were sterile. This slight discoloration of the stem was evidently due to the extremely high soil temperature just previous to digging, as previously mentioned. The inoculated plants started to wilt about April 25, and all 3 inoculated plants at 80 per cent of the moisture holding

[1] In the (18° to 36°) set the temperature of the soil, owing to an accident to the regulating device, rose to 45° 3 nights in succession and was lowered to 36°C. each morning during the last 3 days of the experiment.

TABLE 8.—*Relation of soil temperature and moisture to infection of Early Ohio potatoes from soil inoculated with Fusarium oxysporum No. 35*
Experiment 4

Soil temperature	Soil moisture[1]	Treatment of plants	Symptoms: Wilt	Symptoms: Vascular discoloration	Symptoms: Stem rot	Symptoms: Root injury	Cultures of *F. oxysporum*: From roots	Cultures of *F. oxysporum*: From stems
Degrees C.	*Per cent*	Control.....1	—	—	—	—	—	—
18	60	Inoculated 2	—	—	—	—	—	—
		Inoculated 3	—	—	—	—		—
		Inoculated 4	—	—	—	1	—	—
	80	Control.....1	3	—	—	—	—	—
		Inoculated 2	—	—	—	1	—	—
		Inoculated 3	—	—	—	1	—	—
		Inoculated 4	—	—	—	—	—	—
24	60	Control.....1	—	—	—	—	—	
		Inoculated 2	—	—	1	—	—	
		Inoculated 3	—	—	—	1	—	—
		Inoculated 4	—	—	—	—	—	+
	80	Control.....1	2	2	2	2	—	+
		Inoculated 2	—	—	—	1	+	—
		Inoculated 3	—	—	—	1	—	—
		Inoculated 4	—	—	—	—	—	—
28	60	Control.....1	—	—	—	—	—	—
		Inoculated 2	—	—	—	—	—	—
		Inoculated 3	—	2	2	1	—	+
		Inoculated 4	—	—	—	—	—	—
	80	Control.....1	—	—	—	—	—	—
		Inoculated 2	—	1	—	—	—	
		Inoculated 3	1	1	1	1	—	+
		Inoculated 4	—	—	1	—	—	—
32	60	Control.....1	—	—	—	—	—	—
		Inoculated 2	—	—	—	—	—	—
		Inoculated 3	—	—	—	—	—	—
		Inoculated 4	—	—	1	—	—	—
	80	Control.....1	—	—	—	—	—	—
		Inoculated 2	—	—	—	—	—	—
		Inoculated 3	—	—	—	—	—	—
		Inoculated 4	—	—	1	—	—	—
18[2] to 36	60	Control.....1	1	1	—	—	—	0
		Inoculated 2	1	2	1	3	+	+
		Inoculated 3	3	3	3	3	+	+
		Inoculated 4	1	1	—	1	+	—
	80	Control.....1	1	1	—	—	—	0
		Inoculated 2	3	3	3	3	+	0
		Inoculated 3	3	3	3	3	+	+
		Inoculated 4	3	3	3	3	0	0
36 to 18	60	Control.....1	—	—	—	—	—	—
		Inoculated 2	—	—	—	—	—	—
		Inoculated 3	no	germination				
		Inoculated 4	no	germination				
	80	Control.....1	no	germination				
		Inoculated 2	no	germination				
		Inoculated 3	—	—	—	—	—	—
		Inoculated 4	—	—	—	—	—	—

[1] Soil moistures are expressed in per cent of the moisture holding capacity (35 per cent dry weight).
[2] Final notes on the (18° to 36°C.) plants were taken 10 days before the other plants in the experiment.

capacity were completely wilted by May 2, while 1 plant was completely wilted and 2 slightly wilted in the set at 40 per cent moisture. All of these inoculated plants showed definite signs of disease below ground and the causal organism was recovered from either roots or stems in all but one instance. As previously noted, the plants started at 36° were not above ground when the soil temperature was changed to 18°. At the latter temperature, all plants that grew remained healthy.

The only temperature at which uniform results were secured in this experiment was with plants started at 18° and changed to 36°C. In these plants the wilting symptoms and the vascular discoloration of the stem were undoubtedly due in part directly to the high soil temperature as indicated by the controls. In this set the infection appeared to be greater in soil kept at 80 per cent rather than at 60 per cent of the moisture holding capacity.

The effect of wilting due to high temperature with subsequent recovery shows that it is difficult to examine a plant having slight signs of wilt during an experiment either in the field or under control conditions and attempt to determine whether these slight symptoms of disease are due to environmental conditions or to some wilt producing parasite.

EXPERIMENT 5. NOVEMBER 22, 1921, TO FEBRUARY 20, 1922

This experiment was planned to test the pathogenicity of *Fusarium oxysporum* as a soil parasite when both soil temperatures and soil moistures are changed during the course of the experiment.

Methods.—Healthy tubers of the Bliss Triumph variety were used. They were cut and sprouted in moist sand. The sprouts were ¼ inch long when planted and the cut surface was well calloused over. The soil was mixed the same as in the previous experiment and was sterilized for 3 hours at 2½ pounds pressure. It was then inoculated previous to planting with cultures of *Fusarium oxysporum* No. 35 grown on Melilotus stems. The moisture content of the soil at the start of the experiment was 20.4 per cent dry weight. This was 60 per cent of the moisture holding capacity, previously determined to be 34 per cent dry weight. The soil was kept at the desired moisture content as in Experiment 4. All the containers were placed at the desired temperatures immediately after planting. Eighty plants were grown in the experiment, 60 of them in inoculated soil and 20 used as controls. Forty of these plants were started in a cool greenhouse at a temperature of about 18° C. Thermograph records of the air temperature in the house showed an average mean of 64.7° F. (18.2° C.), an average maximum of 69.3° F. (20.7° C.), and an average daily minimum of 60.1° F., (15.6° C.). The other 40 plants were started in 5 soil temperature tanks in the same greenhouse. These tanks were held at 24°, 28°, 28°, 32°, and 36° C.

Temperature records were made twice daily in all the tanks and

showed the temperature of the soil in the various sets at the depth of 3 inches to average 23.6°, 27.7°, 27.9°,30.6°, and 35.7° C.

On January 3 all the plants growing in tanks at 24°, 28°, 32°, and 36° were transferred to room temperature (18° C.). Thirty-two of the plants that had been growing at (18°) room temperature were transferred to 24°, 28°, 32°, and 36° tanks. One set of 8 plants was allowed to remain in the second 28° tank thruout the experiment and another set was left at (18°) room temperature thruout the experiment. At the time the temperatures were changed, January 3, the soil moisture of half of the plants in each set, 3 inoculated and 1 control, was allowed to drop to 13.6 per cent dry weight, or 40 per cent of the moisture holding capacity; the other half of each set remaining at 60 per cent of the moisture holding capacity.

Results.—All plants were examined almost daily and the notes taken are represented briefly in Tables 9a and 9b with the final notes taken at digging time.

At the time the temperatures were changed (January 3) all the plants growing at 18°C. (room temperature) were in a healthy, vigorous condition. The plants in the tanks were all healthy and vigorous except at 32° and 36°, where the high soil temperature caused rather small, weak, spindling plants. When these plants were changed to 18° they gradually lost this appearance, and at the end of the experiment were apparently not suffering from this previous exposure to a high soil temperature. Likewise, the plants changed from 18° to 32° and 36° turned yellow and wilted considerably during the first 2 weeks, but after losing their lower leaves growth was renewed and the controls showed no signs of wilting at the close of the experiment. The effect of the dropping of the soil moisture content from 60 to 40 per cent of the moisture holding capacity of the soil was apparent in a drooping and wilting of the leaves. This was not accompanied by any yellowing, however, and the plants usually recovered from the change in the soil moisture in less than 2 weeks. Only 3 control plants out of 20 in the experiment showed any wilt. Isolations were made from both stems and roots of these control plants, and *Fusarium oxysporum* was not recovered in any instance.

The greatest amount of infection occurred at the constant temperature of 28°C. There was severe wilting of all plants, accompanied by a vascular discoloration and considerable root injury and isolations revealed the presence of *Fusarium oxysporum* in every plant. There was no apparent difference in the amount of wilt at this temperature with different soil moisture contents. The next greatest amount of wilting occurred in the plants grown at 18° and changed to 32° and 36° with 40 per cent

TABLE 9a.—*Relation of varying soil temperatures and moistures to the infection of Bliss Triumph potatoes from soil inoculated with Fusarium oxysporum. No. 35*

Experiment 5

Soil moisture[1]	Temperature	Treatment of plants		Symptoms				Cultures of *F. oxysporum*	
				Wilt	Vascular discoloration	Stem rot	Root injury	From roots	From stems
Per cent	*Degrees C.*								
	18				All plants healthy				
	18 to 24	Control	1	—	1	—	1	—	0
		Inoculated	2	—	1	—	1	—	0
			3	—	—	—	—	—	—
			4	—	—	—	—	—	—
	18 to 28	Control	1	—	1	—	—	—	0
		Inoculated	2	—	—	—	—	—	—
			3	—	1	—	—	—	—
			4	—	—	—	1	—	—
	18 to 32	Control	1	—	1	—	1	0	—
		Inoculated	2	2	—	—	—	—	—
			3	3	1	—	2	+	+
			4	—	—	—	—	—	—
	18 to 36	Control	1	—	3	—	1	0	0
		Inoculated	2	3	3	3	3	dead	dead
			3	3	1	—	1	0	0
			4	—	—	—	—	—	
60 to 40	36 to 18	Control	1	—	—	—	—	—	—
		Inoculated	2	—	—	—	—	—	—
			3	—	3	3	1	—	+
			4	—	—	—	—	—	—
	32 to 18	Control	1	—	1	—	—	—	—
		Inoculated	2	—	—	—	—	—	—
			3	—	1	—	1	+	—
			4	—	1	—	1	+	+
	28 to 18	Control	1	3	3	—	1	0	0
		Inoculated	2	—	—	—	—	—	
			3	—	3	—	1	0	—
			4	—	—	—	—	—	—
	24 to 18	Control	1	—	—	—	—	—	—
		Inoculated	2	—	—	—	—	—	—
			3	—	—	—	—	—	—
			4	—	1	—	—	0	+
	28	Control	1	3	3	—	3	0	0
		Inoculated	2	1	3	2	3	+	+
			3	3	3	—	2	+	+
			4	3	3	—	3	0	+

[1] Soil moistures are expressed in per cent of the moisture holding capacity (34 per cent dry weight).

soil moisture. The only other wilted plants occurred at (18° to 28°) and (28° to 18°). All plants held at 18° remained healthy.

TABLE 9b.—*Relation of varying soil temperatures and moistures to the infection of Bliss Triumph potatoes from soil inoculated with Fusarium oxysporum No. 35*

Experiment 5

Soil moisture[1]	Temperature	Treatment of plants	Symptoms: Wilt	Symptoms: Vascular discoloration	Symptoms: Stem rot	Symptoms: Root injury	Cultures of *F. oxysporum*: From roots	Cultures of *F. oxysporum*: From stems
Per cent	*Degrees C.*							
	18			All plants healthy				
	18 to 24	Control.....1	—	1	—	1	0	0
		Inoculated 2	—	3	—	2	0	+
		3	—	3	—	1	0	+
		4	—	—	—	—	—	—
	18 to 28	Control.....1	—	1	—	—	—	+
		Inoculated 2	1	3	2	3	—	0
		3	1	—	—	—	—	—
		4	—	3	1	1	0	0
	18 to 32	Control.....1	—	—	—	—	—	—
		Inoculated 2	—	3	—	2	+	+
		3	—	2	3	3	0	0
		4	—	—	—	—	—	—
	18 to 36	Control.....1	—	1	—	2	0	+
		Inoculated 2	—	3	2	2	0	0
		3	—	—	—	1	0	—
		4	—	3	—	2	+	+
60 to 60	36 to 18	Control.....1	—	2	1	1	—	0
		Inoculated 2	—	—	—	—	—	—
		3	—	—	—	—	—	—
		4	—	—	—	—	—	—
	32 to 18	Control.....1	—	—	—	—	—	—
		Inoculated 2	—	—	—	—	—	—
		3	—	—	—	—	—	—
		4	—	3	—	2	+	+
	28 to 18	Control.....1	—	—	—	—	—	—
		Inoculated 2	—	—	—	1	—	—
		3	—	—	—	—	—	—
		4	—	2	—	1	0	0
	24 to 18	Control.....1	—	—	—	—	—	—
		Inoculated 2	—	—	—	—	—	—
		3	—	—	—	—	—	—
		4	—	—	—	—	—	—
	28	Control.....1	3	2	—	2	0	0
		Inoculated 2	2	3	—	3	+	+
		3	1	3	3	3	+	0
		4	3	3	2	3	+	+

[1] Soil moistures are expressed in per cent of the moisture holding capacity (34 per cent dry weight).

There was a very slight amount of disease present in the plants grown at (18° to 24°) and (24° to 18°). No wilting occurred

in any of the inoculated plants changed to 18° on January 3 altho vascular discoloration was present in a number of plants and the causal organism was isolated in pure culture.

Isolations were made from practically all the plants showing wilting accompanied by vascular discoloration or considerable root injury. One plant at (18° to 36°C.) was so badly decayed that isolations were impractical. In most cases 4 tissue cultures were made from each specimen. If any of these showed *Fusarium oxysporum* the cultures were marked in the table as positive. From 28 plants with diseased roots, *F. oxysporum* was isolated 11 times, or 39 per cent. Cultures from the stems of 32 plants yielded *F. oxysporum* 16 times or 50 per cent. Fifteen plants showed complete or nearly complete wilt. *F. oxysporum* was isolated from only 7 of them. Two of these 15 plants showed no vascular discoloration or root injury and the other 6 yielded various species of Fusaria, but not *F. oxysporum.* Slight yellowing of the lower leaves even when accompanied by slight wilting appears to have no value in estimating the amount of infection.

The chief difference in the effect of changing the soil moisture at the time the temperatures were changed appeared to be in the greater amount of stem rot and the smaller amount of wilt with the higher soil moisture. The extent of root injury was greater on infected plants at the higher moisture content altho there was only one more plant showing root injury than at the low moisture.

There was undoubtedly some vascular discoloration, either accompanying positive wilting symptoms or without any signs of wilt, that was not caused by *Fusarium oxysporum.* Cultures made from 20 plants showing vascular discoloration accompanied by wilt yielded *F. oxysporum* only 10 times; and 12 plants showing vascular discoloration and with no wilt yielded *F. oxysporum* only 6 times. Thus, the causal organism was isolated from only 50 per cent of the plants. Neither vascular browning nor wilting of the tops is a sure index of the presence of *F. oxysporum.*

EXPERIMENT 6, FEBRUARY 13 TO MAY 11, 1922

This experiment differs from the previous soil temperature experiment in that the soil used was not sterilized and the freshly cut seed tubers were dipped in a water suspension of spores just previous to planting. The soil was also inoculated as in the previous experiments.

It was thought that soil sterilization or disinfection might have been a factor in causing the small percentage of positive infections obtained in previous experiments, so this experiment

was planned in order to eliminate such possible injury. The seed-piece as well as the soil was inoculated in an attempt to produce a type of infection similar to that reported by MacMillan (1919). Infection thru the seed-piece from the soil did not appear to any extent in the previous experiments when the soil was heavily inoculated, so this experiment was planned with a direct inoculation of the freshly cut seed-piece as well as soil inoculation.

Methods.—The soil was mixed as in the previous experiments and inoculated with cultures of *Fusarium oxysporum* No. 35 grown on boiled rice and Melilotus stems. The seed tubers used were from the same lot as those in Experiment 5. The freshly cut seed-pieces were dipped in a water suspension of spores similar to the inoculum used for the soil. The soil moisture was held at 26.3 per cent dry weight. This was 70 per cent of the moisture holding capacity, previously determined to be 37.6 per cent dry weight. Immediately after planting, all cans were removed to the desired temperatures. The cans were watered thruout the experiment by surface and subsoil watering. The surface of the soil was covered with crushed rock. Six inoculated and 2 control cans were used at each of the following temperatures, 18°, 24°, 28°, 32°, and 36°C. Temperature records showed the temperature in the various sets to average as follows: 17.9°, 24.1°, 28.2°, 32.0°, and 34.7°. The air temperature was held at 18° at the start, but during the latter half of the experiment it often reached 25° during the day.

Results.—Altho we would expect a greater amount of infection in this experiment than in Experiment 5, due to the direct inoculation of freshly cut seed, Table 10 shows a very small percentage of positive inoculations. The 2 experiments were run for the same length of time and inoculum from the same strain of Fusarium was used. Potato seed from the same source was used in both experiments, but the seed in this experiment was about 3 months older than in Experiment 5. At the close of the experiment, only one typical case of wilt due to infection with *Fusarium oxysporum* was found. In general, there was considerable drying and yellowing of the leaves, due to the rather high air temperature toward the close of the experiment. The plants at 18°C. were the most vigorous in the experiment. The greatest difference between inoculated and control plants was found in the few plants grown at 32°. The only positive case of wilt occurred at 28°. In general, the controls showed as much infection as the inoculated plants. This was probably due to the fact that the soil was not sterilized. Three control plants and only 2 inoculated plants showed vascular discoloration of the underground stem, altho the infection appeared deeper in the inoculated ones than in the controls. Three controls and 4 inoculated plants showed root injury, and again the injury appeared greatest in the in-

TABLE 10.—*Relation of soil temperature to infection of Bliss Triumph potatoes when both soil and seed were inoculated with Fusarium oxysporum No. 35*

Experiment 6 [1]

Temperature	Treatment of plants		Symptoms: Wilt	Symptoms: Vascular discoloration	Symptoms: Stem rot	Symptoms: Root injury	Cultures of *F. oxysporum*: From roots	Cultures of *F. oxysporum*: From stems
Degrees C.								
18	2 controls, 6 inoculated plants, all healthy							
24	Control	1	—	2	—	2	+	+
		2	—	1	—	2	+	+
	Inoculated	3	—	—	—	—	—	—
		4	—	—	—	—	—	—
		5	—	—	—	—	—	—
		6	—	—	—	—	—	—
		7	—	—	—	—	—	—
		8	—	—	—	1	—	—
28	Control	1	—	1	—	2	+	+
		2	—	—	—	—	—	—
	Inoculated	3	—	—	—	2	—	—
		4	—	—	—	1	—	—
		5	—	—	—	—	—	—
		6	—	—	—	—	—	—
		7	3	3	2	3	+	+
		8	—	—	—	—	—	—
32	2 controls and 5 inoculated plants failed to germinate							
	Inoculated	8	—	2	2	3	+	+
18[2] to 32	Control	1	—	—		—	—	—
	Inoculated	2	—	—		2	—	—
36	2 controls and 6 inoculated plants failed to germinate							

[1] The moisture content of the soil was kept at 70 per cent of the moisture holding capacity or 26.3 per cent dry weight.

[2] These two plants had been growing at 18° and were placed at 32°C. on March 22 to replace seed that failed to germinate.

oculated ones. Cultures of *F. oxysporum* were obtained from all plants showing vascular browning of the underground stem.

Effect of High Soil Temperature on the Potato Plant

Two plants inoculated with *Fusarium oxysporum* and 1 control plant grown in the 18° tank with an air temperature of about 18° C. were found to be healthy at the end of the experiment. These plants were left in the tanks and were submitted to the extreme soil temperature of about 60° for one hour and then brought back to 18° and left at this temperature for 2 weeks. All 3 plants when dug showed a distinct vascular browning, appearing very similar to the vascular browning caused by *F. oxysporum*. The tissue of the underground portion of the stem was softened and slightly rotted both in the inoculated and in the control plants. The roots were slightly injured. Cultures from the browned vascular system were sterile. This would indicate that we might expect vascular browning from other causes than *F. oxysporum* infection, and that this browning is rather a poor criterion for pronouncing the plant to be infected with *F. oxysporum* unless it is considered along with other symptoms. While the temperature used was extremely high, it is very probable that the same results may be obtained by using a lower temperature with a longer or intermittant exposure.

Effect of High Air and Soil Temperature on the Disease

Six pots, 4 with inoculated soil and 2 controls, were planted at the same time as Soil Temperature Experiment 1. Similar soil and seed and the same methods were used for the inoculations. These pots were kept in a greenhouse maintained at the approximate temperature of 25° C. until they were all about 6 inches high with a good even growth and no signs of disease. They were then removed to a high temperature greenhouse (30°-33°). Ten days after being changed to this high temperature the infected plants were badly yellowed and wilted; the controls were slightly wilted. Ten days later, all the plants were entirely wilted. The controls, altho entirely dead above ground, showed no signs of disease in the underground parts, the stems, roots and stolons being healthy. *Fusarium oxysporum* was recovered from the rotted underground portion of the stems of the inoculated plants.

It was very probable that infection had already set in before these plants were removed to the high temperature and that this high temperature simply favored the rapid advance of the disease. It is noticeable, however, that altho the roots and stems of the control plants remained healthy the tops wilted rapidly, and the above-ground symptoms could not be used in identifying the presence of the organism.

Relation of Soil Moisture to Infection of Roots from the Soil

EXPERIMENT 1, DECEMBER 11, 1920, TO MARCH 11, 1921

In the previous experiments with controlled soil temperatures it was found that the soil moisture content was also a factor in determining the amount and severity of infection and that a change in the soil moisture during the experiment affected the results. The extent of the influence of soil moisture was rather hard to determine, as the soil temperatures were also varied in these experiments. The following experiment was planned to test the effect of varying soil moisture contents upon the disease when the soil and air temperature remained approximately constant.

Methods.—The experiment was conducted in a greenhouse maintained at a temperature of approximately 25°C. Seed tubers of the Early Ohio variety were used from the same lot as those used in Soil Temperature Experiment 3, and were treated in the same way. Soil was prepared in the same way as for the soil temperature experiments. Four-gallon earthenware crocks were used for containers, each holding 16,650 grams of soil, dry weight. The soil was inoculated just previous to planting with cultures of *Fusarium oxysporum* No. 35. Two seed tubers were planted in each container. The soil moisture was maintained by watering methods similar to those used in the soil temperature experiments. One thousand grams of crushed rock was used on the surface of the soil. The moisture holding capacity of the soil as determined by the 25 cm. cup method was 35 per cent dry weight. Twenty-seven containers were used, being divided into 3 sets, each set composed of 3 controls and 6 inoculated pots. The soil in these 3 sets of 9 containers each was started at approximately 40, 60, and 80 per cent of the moisture holding capacity of the soil, equal to 14, 21, and 28 per cent dry weight, respectively. The soil after inoculation was brought up to the desired moisture content in each set and allowed to stand about 10 days before planting. The soil moisture content in each of these sets was changed on January 8 by dividing each set into 3 lots of 1 control and 2 inoculated containers each. Thus, in Set 1, 9 containers (at 40 per cent of moisture holding capacity) were divided into 3 lots. Three containers were left at 40 per cent, 3 changed to 60 per cent, and 3 to 80 per cent of the moisture holding capacity of the soil. In the same way, the sets being held at 60 and 80 per cent were each subdivided into 3 lots. In changing the moisture contents, if the desired point was higher, water was added at the rate of 500 c. c. per day until the desired point was reached. If a lower moisture content was wanted, the soil was allowed to dry out naturally until the desired moisture content was reached. The maximum change, that is, from 40 to 80 per cent of the moisture holding capacity or vice versa, took place in approximately 10 days. From 40 to 60 per cent or 60 to 80 per cent took about one-half this time. Weighings were made and the moisture contents calculated for each day while changing, but as the slight variation in the length of time necessary to change the moisture content did not appear to be correlated with any of the final results, the figures are not presented here. On February 15 the moisture contents of the 9 lots were changed back to the original moisture content of the 3 sets.

TABLE 11.—*Relation of soil moisture to infection of Early Ohio potato plants from soil inoculated with Fusarium oxysporum No. 35*

Experiment 1[1]

Soil moisture[2]	Treatment of plants	Symptoms: Wilt	Symptoms: Vascular discoloration	Symptoms: Stem rot	Symptoms: Root injury	Cultures of *F. oxysporum*: From roots	Cultures of *F. oxysporum*: From stems
Per cent							
40	Control.....1	—	—	—	—	—	—
	Inoculated..2	—	—	—	—	—	—
	Inoculated..3	—	—	1	1	+	+
40 to 60 to 40	Control.....1	—	—	1	1	—	+
	Inoculated..2	3	3	3	3	+	+
	Inoculated..3	2	1	—	1	+	+
40 to 80 to 40	Control.....1	1	2	1	1	—	—
	Inoculated..2	1	3	1	3	—	+
	Inoculated..3	2	3	3	3	+	+
60	Control.....1	—	—	—	—	—	—
	Inoculated..2	—	1	—	—	—	+
	Inoculated..3	—	—	1	1	—	+
60 to 40 to 60	Control.....1	—	—	—	—	—	—
	Inoculated..2	1	—	—	1	—	—
	Inoculated..3	—	1	1	—	—	+
60 to 80 to 60	Control.....1	—	—	—	—	—	—
	Inoculated..2	—	3	3	—	—	+
	Inoculated..3	—	—	1	—	—	+
80	Control.....1	1	—	—	—	—	—
	Inoculated..2	1	2	2	1	—	+
	Inoculated..3	—	—	—	—	—	—
80 to 60 to 80	Control.....1	1	—	—	—	—	—
	Inoculated..2	1	3	3	—	—	+
	Inoculated..3	—	1	—	—	—	+
80 to 40 to 80	Control.....1	—	—	1	1	—	+
	Inoculated..2	—	—	—	—	—	—
	Inoculated..3	2	2	3	—	—	+

[1] Each container represents 2 plants, being considered here as a unit. If either plant in a pot became infected the plant is marked positive, the degree of infection being based on the number of stems and roots infected and the severity of infection.

[2] Soil moistures are expressed in per cent of the moisture holding capacity (35 per cent dry weight).

Results.—The results of this experiment are shown in Table 11. The germination of the seed was very uniform, altho the plants started at 40 per cent of the moisture holding capacity were a little slower in breaking thru the ground. On January 8, when the soil moistures were first changed, all the plants were healthy altho the size varied with the moisture content of the soil, those growing at 40 per cent averaging about 2.5 inches in height, those at 60 per cent, 4 inches, and those at 80 per cent, 4.5 inches. The change in soil moistures, where the moisture content was lowered, was sometimes followed by a drooping of the leaves, but the plants recovered quickly and were apparently normal in about 10 days, the time used in reaching the new moisture content. On February 15, when the moisture contents were again changed, no definite wilting had occurred in any of the plants, altho there was a slight yellowing of the lower leaves in all sets, this being more marked in the 40 per cent set than at the higher moisture contents. At the higher moistures, however, the plants were not as sturdy, the tops often drooping over. At the close of the experiment it was found that the underground stems of the plants at 80 per cent were much smaller in diameter than those at the lower moisture contents. As shown in Table 11, the sets held at a constant moisture content showed less infection than those in which the moisture content was changed. The smallest amount of infection occurred at the constant moisture content of 40 per cent while the largest amount occurred at 80 per cent. The reverse is true, however, in regard to the pots in which the moisture content was varied. The largest amount of wilt along with the heaviest infection of stem and roots occurred in the (40 to 80 to 40) per cent lot, and the next in the (40 to 60 to 40) per cent lot. There was not a great deal of difference between the other sets, altho there appeared to be slightly more stem infection in the 80 per cent lots than in the 60 per cent lots. Three of the control plants showed symptoms of the disease but *Fusarium oxysporum* was isolated successfully from only one of them. Cultures from the inoculated plants showing symptoms of disease yielded *F. oxysporum* from either the stems or the roots in every instance.

EXPERIMENT 2, DECEMBER 5, 1921, TO FEBRUARY 20, 1922

This experiment was planned somewhat similarly to Soil Moisture Experiment 1, except that a different variety of potatoes was used and the soil moistures were changed once, and were not brought back to the original soil moisture content as in Experiment 1.

Methods.—Tubers of the Bliss Triumph variety were used from the same lot as those used in Soil Temperature Experiment 5. They were treated in the same way, except that immediately before planting the cut surfaces which had calloused over while the sprouts were starting were freshly cut and planted in soil that had been inoculated at the same time and in the same way as for Soil Temperature Experiment 5. The tubers were planted in 3-gallon stone crocks, one seed-piece to each crock which contained 12 kilograms of soil with 1,000 grams of gravel added to the surface. The soil was watered as in the previous experiment. All the pots were filled with soil and allowed to stand for 2 weeks before planting. During this time the moisture content of the soil was brought up to the desired point. Thirty-six crocks were used in the experiment, being divided at the start into 3 sets, each having 9 inoculated and 3 control crocks. These 3 sets were held at 3 different soil moisture contents, as indicated in Table 12. The moisture holding capacity of the soil was 34 per cent dry weight determined by the 25 cm. cup method. Set 1 was held at 40 per cent of the moisture holding capacity (12.1 per cent dry weight); set 2 at 60 per cent (20.4 per cent dry weight); set 3 at 80 per cent (27.2 per cent dry weight). When the plants were about 6 inches in height, January 9, the soil moisture was changed, so that each set was divided up into 3 lots, each with 3 inoculated and 1 control plant, the same as in the previous experiment. The entire experiment was run in a greenhouse where thermograph records showed the average daily maximum temperature to be 80.5°F. (26.9°C.), the average daily minimum at 71.5°F. (21.9°C.), and the average daily mean temperature 75.5°F. (24.2°C.).

In order to check the possible error in the percentage of soil moisture in the different sets, 2 samples were taken from each of 3 crocks labeled 40, 60, and 80 per cent of moisture holding capacity. These showed the following errors to be present at the end of the experiment in the pots tested:

40 per cent = 12.1 per cent dry weight, at close of experiment was 41.1 per cent or 14 per cent dry weight.

60 per cent = 20.4 per cent dry weight, at close of experiment was 60.5 per cent or 20.6 per cent dry weight.

80 per cent = 27.2 per cent dry weight, at close of experiment was 70.0 per cent or 23.8 per cent dry weight.

This showed practically no error in the crocks at 40 and 60 per cent of the moisture holding capacity, but a considerable drop in the 80 per cent set. This was probably due to the increased weight of the plant which was not taken into account in the watering and which in the 40 to 60 per cent sets seemed to be about balanced by the decrease in the moisture holding capacity of the soil due to packing during the experiment.

Results.—All plants were dug and examined February 20, 1922. Notes were taken thruout the experiment. All plants remained healthy except for a drying and slight yellowing of the lower leaves during the latter part of the experiment. No typical Fusarium wilt appeared in any of the plants.

The tabulated results in Table 12 do not show any clear-cut results as to the effect of soil moisture on the disease. One plant

died at 40 per cent moisture in Set 1, but this appeared to be due to causes other than *Fusarium oxysporum*.

As in the previous experiment, the plants were about one week slower in breaking thru the ground at 40 per cent than at the higher moisture contents. When the soil moistures were changed, all the plants in the experiment were apparently healthy and averaged about 6 inches in height. At the close of the experiment all plants showed a slight yellowing and drying of the

TABLE 12.—*Relation of soil moisture to infection of Bliss Triumph potatoes from soil inoculated with Fusarium oxysporum No. 35*

Experiment 2

Soil moisture[1]		Treatment of plants		Symptoms				Cultures of *F. oxysporum*	
				Wilt	Vascular discoloration	Stem rot	Root injury	From roots	From stems
Per cent									
40	40	Control	1	—	1	—	—	—	0
		Inoculated	2	—	—	—	—	—	—
			3	—	—			0	0
			4	—	—	—	—	—	—
	60	Control	1	—	—	—	—	—	—
		Inoculated	2	—	—	—	—	—	—
			3	—	1	—	1	+	+
			4	—	—	—	—	—	—
	80	Control	1	—	1	—	—	—	0
		Inoculated	2	—	—	—	—	—	—
			3	—	—	—	—	—	—
			4	—	—	—	—	—	—
60	40	Control	1	—	—	—	—	—	—
		Inoculated	2	—	2	2	1	0	+
			3	—	1	—	1	—	—
			4	—	—	—	—	—	—
	60	Control	1	—	—	—	—	—	—
		Inoculated	2	—	1	—	—	—	—
			3	—	—	—	—	—	—
			4	—	—	—	—	—	—
	80	Control	1	—	—	—	—	—	—
		Inoculated	2	—	—	—	—	—	—
			3	—	1	—	—	—	0
			4	1	—	—	—	—	—
80	40	Control	1	—	—	—	—	—	—
		Inoculated	2	—	1	—	1	+	+
			3	—	—	—	—	—	—
			4	—	—	—	—	—	—
	60	1 control, 3 inoculated healthy							
	80	1 control, 3 inoculated healthy							

[1] Soil moistures are expressed in per cent of the moisture holding capacity, (34 per cent dry weight).

lower leaves not associated with the disease. There were no marked differences between the various sets. Only one plant showed any wilting symptoms and this was not due to Fusarium infection.

It is worthy of note that altho this experiment was conducted at a temperature averaging about 25° and the same inoculated soil, and seed from the same source as that of Soil Temperature Experiment 5 was used, the amount of infection was considerably less than that obtained in the Soil Temperature Experiment at a constant temperature of 28°C., where the greatest amount of infection took place.

It is also noticeable that in this experiment where the soil was heavily infested the seed tubers were planted with a freshly cut surface exposed for infection and at a temperature and soil moisture higher than that found under ordinary field conditions, and yet no results were obtained that were remotely comparable with those reported by MacMillan (1919) for soil infection thru the seed-pieces under field conditions.

DISCUSSION

These experiments, conducted with as many environmental factors under control as was possible and with inoculations with various strains of *Fusarium oxysporum*, show the usual confusion of results. It was impossible to obtain consistent results in consecutive experiments and in most cases the percentage of infection was rather low. It was evident, however, that temperatures of 18°C. and below were unfavorable for the development of the disease and that in general the amount of infection increased with increasing soil temperatures. It was noticeable that under these conditions the disease developed most readily at temperatures unfavorable for the host plant. Plants started at a temperature low enough to be unfavorable for the disease but later transferred to a higher temperature showed nearly as much disease as those kept at a constant high temperature; while those started at high temperatures and later transferred to lower temperatures showed practically no disease, as did those kept at a constant low temperature.

Constant low soil moistures were unfavorable for the development of the disease. However, when the soil moisture content was varied it was found that the disease developed best when the plants were started at a low soil moisture content, changed to high soil moistures during the critical period in the life of the plant, and later lowered to the original low soil moisture content.

It was also noticeable in all these tests that with increasing soil moisture the amount of rotting of the roots and stems increased and the wilting symptoms were not as marked as at the lower soil moistures. It was also shown that there was often present a discoloration of the vascular system of the main stem, either in the absence of wilt or accompanying these symptoms, that was not caused by *Fusarium oxysporum*. If the plants were submitted to extremely high temperatures for relatively short intervals. a vascular discoloration was produced that had all the aspects of Fusarium infection.

It is also worthy of note that in all these infection tests no correlation could be made between the presence of the disease and the rotting of seed-pieces. Where infection took place, it was thru the roots and not thru the seed-pieces. In all of these tests, the seed-pieces behaved alike both in the controls and in the inoculated plants.

INFECTION FROM THE SOIL THRU THE SEED-PIECE

Review

In all the published work on Fusarium wilt previous to 1919, the emphasis was placed upon the infection of the plant from the soil thru the roots or by the planting of infected seed. Infection of the plant from the soil thru the seed-piece was not recorded until MacMillan's (1919) publication. This may very probably be accounted for as MacMillan suggests (p. 280):

> Since the part of Fusarium spp. in the creation of disease depends largely on environmental factors, it is important to note that the conditions which prevail in Colorado do not exist in the same way in other places. The descriptions of diseases caused by species of Fusarium already published do not, therefore, closely apply to Colorado, because the climate, coupled with the soil conditions and irrigation practice existing there, creates a condition unknown in the East, where most of the work on Fusarium spp. has been done.

The writer has found this statement to hold true not only as regards the different manifestations of the disease in the irrigated sections of western Nebraska as compared with previous observations in Michigan, but also as compared with dry land sections of western Nebraska. In other words, the type of infection appears to be due in a great measure to the environmental conditions and it is impossible to predict what will happen under irrigation in the West from experimental data obtained in the East or from dry land conditions in the West. It is not necessary, however, to have a different type of infection in order to produce

different symptoms of disease; it is only necessary to have present different environmental conditions after infection has taken place. All 3 types of infection discussed in this paper have been found in Michigan as well as in the irrigated fields of Nebraska but the amount of each of these 3 types varied with the conditions present.

MacMillan reports experiments in which the seed tubers were inoculated with a spore suspension of *Fusarium oxysporum* and then planted either in flats or under field conditions, also by inoculating the seed-pieces of plants which had already sent up sprouts. He obtained nearly 100 per cent infection with this method of inoculation. He concludes from these experiments (p. 300):

> Two methods of infection are recognized: Infection from the soil of roots and root hairs, and infection of the seed-piece, whereby the plant becomes diseased. The latter method is regarded as the most serious and responsive to environmental conditions in the Greeley district of Colorado.

While he does not state the conditions under which his experiments were conducted as to temperature and moisture, he says in referring to the relation of soil temperature to infection (p. 299):

> Seed-piece infection will occur at a considerably lower temperature than root infection.

The author found that under the conditions prevailing in Michigan during the experiments previously discussed infection from the soil thru the seed-pieces occurred but rarely. It was found so seldom that it was not considered of any importance under those conditions. It might also be noted that in these experiments rotting of the underground stem was very rare, and in general, conditions were not favorable and probably are very seldom favorable in Michigan for this type of infection. In the numerous soil infection experiments conducted under controlled conditions, the seed-piece very often remained healthy thruout the experiment and in only a few cases could the infection be tracd to the seed tuber.

Greenhouse Experiments, 1920-21

In 1920 a few tests were made with artificially infected seed. Healthy Irish Cobbler potatoes were inoculated by placing a few drops of spore suspension in a small hole bored out of the tuber. It was then sealed with a cover glass and planted in sterilized soil. Three seed-pieces inoculated with *Fusarium oxysporum* No. 33 and grown in a greenhouse maintained at approximately 17°C. produced healthy plants with no signs of wilt. One seed-piece had rotted slightly and the base of one stem of this plant was slightly infected. In the other 2 the infection of the seed-piece

progressed only a short distance beyond the point of inoculation and the seed remained attached to the stem when the plant was dug. Two seed-pieces were inoculated with *F. oxysporum* No. 8 and kept at the same temperature. Both plants were healthy, the seed-pieces being only slightly rotted. All these plants were duplicated in another greenhouse with a temperature maintained at approximately 27°. All plants remained healthy, and the seed-pieces were still attached, altho they had rotted considerably further than in the plants held at 17°.

During 1921, 16 tubers were inoculated in the same way with *Fusarium oxysporum* No. 35, and kept in a greenhouse at a temperature of 25°C. Half of these plants were watered as usual and the other half were given an excess of water. Only 1 plant showed wilt and this was in the heavily watered set. The seed-piece had completely decayed and the infection of the main stem started at the base and had resulted in a soft rot.

This method of infection was also tried in Soil Temperature Experiment 6, p. 58, where the freshly cut seed-pieces were dipped in a spore suspension just previous to planting, the soil also being inoculated.

Field Experiments, 1921

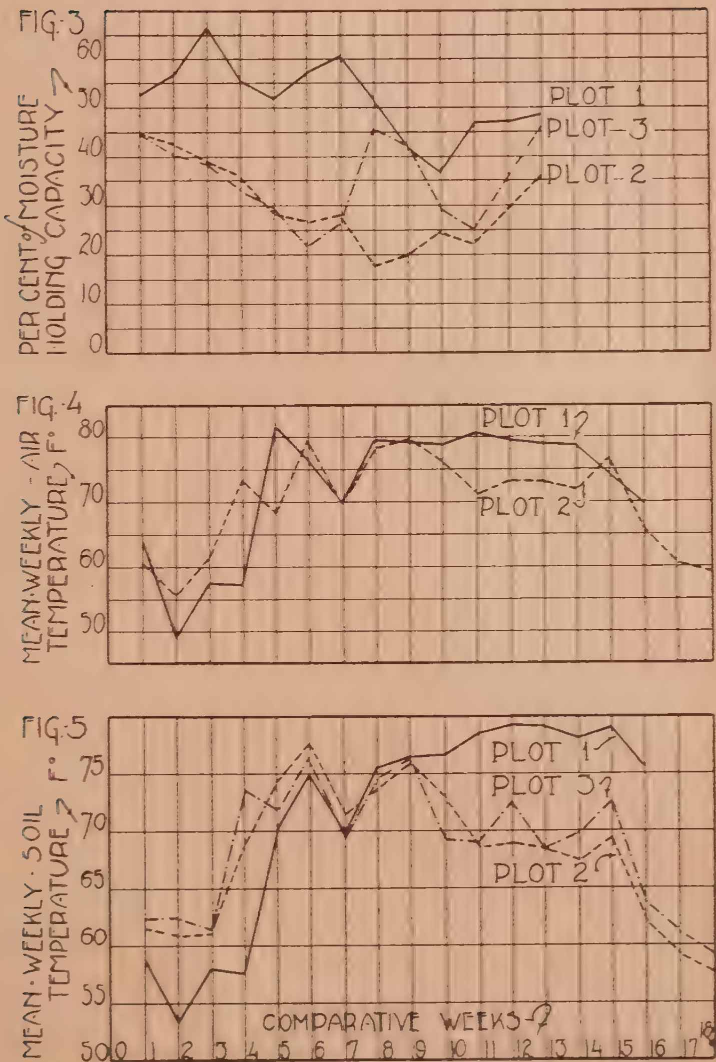
This method of infection was tested in 1921 under field conditions. The experiment was run in triplicate, three plots having different environmental conditions being used.

Location of plots.—Plot 1 was located at the Agricultural College, Lincoln, Nebraska. No potatoes had been grown on this land for 15 years. This plot was used to represent the conditions found in eastern Nebraska, where there is an average annual rainfall of about 30 inches and a high mean daily temperature during the summer months. Plot 2 was located at the substation at Mitchell, Nebraska. The soil was a light sandy loam and was handled as a dry land plot. Plot 3 was located also at Mitchell, but on a heavier soil, and the crop was grown under irrigation. Plots 2 and 3 were used to represent the dry land and irrigated potato sections of western Nebraska, where the annual rainfall is only about 16 inches, or half that of eastern Nebraska, and where the daily mean temperatures are lower than eastern Nebraska, and the night temperatures considerably lower. The meteorological data for these 3 plots are shown in Figures 3, 4, and 5. Neither Plot 2 nor 3 was under the true typical conditions found in most of western Nebraska, but they approximated it as closely as was practical.

Rainfall.—There was considerable difference in the precipitation records between eastern Nebraska and western Nebraska. During the months that the experiment was conducted, we find the following monthly precipitation records.

	April	May	June	July	Aug.	Sept.
Lincoln	2.27 in.	3.44 in.	4.95 in.	6.00 in.	3.35 in.	3.63 in.
Mitchell	0.44 in.	2.32 in.	1.84 in.	2.40 in.	1.79 in.	1.38 in.

During the time the experiment was conducted at Lincoln, that is, April 20 to August 8, there were 23 days with more than 0.1 inch precipitation, and there were only 4 periods of more than 5 days without measurable precipitation, the longest being 10 days. There were 5 rainfalls of more than 1 inch.



FIGS. 3, 4, and 5.—Graphs showing soil moisture (Fig. 3) for foot depths expressed as per cent of the moisture holding capacity, mean weekly air temperature (Fig. 4) and soil temperature (Fig. 5) for comparative weeks of 3 different plots in the 1921 Field Experiments.

During the experiment at Mitchell, May 20 to September 20, a period about 2 weeks longer than the Lincoln Experiment, there were 21 days with more than 0.1 inch precipitation. There were 6 periods of more than 5 days without precipitation, one being for 15 days and two for 11. During this period there were no rainfalls of 1 inch or more.

Soil moisture.—The soil moisture of the various plots is represented graphically in Figure 3. The soil samples were obtained with a soil auger for 1 ft. depths. The moisture holding capacity of the various plots, obtained by the 25 cm. cup method, was as follows: Plot 1, 50 per cent dry weight; Plot 2, 30 per cent dry weight; Plot 3, 35 per cent dry weight. The graphs in Figure 3 are based upon the percentage of the moisture holding capacity rather than upon the actual percentage of the moisture by dry weight.

It is clearly seen that Plot 1 had a higher percentage of moisture throughout the experiment. There was a considerable drop in moisture about 3 weeks before the close of the experiment. Plots 2 and 3 had about the same relative amounts of moisture for the first month of the experiment, after which Plot 3 shows an increase in moisture due to irrigation. Two applications of water were made to Plot 3, the first on July 20, the second on August 12. The results of these irrigations are clearly shown in the graphs.

Air temperature.—In Figure 4 are presented the weekly mean air temperatures for comparative weeks of the experiment for all 3 plots. During the first 4 weeks of the experiment the air temperatures were considerably lower in Plot 1 than in the others; the opposite was true during the latter part of the experiment. The air temperatures of Plots 2 and 3 were about the same since these were located within a short distance of each other; accordingly, only Plot 2 is represented in Figure 4.

Soil temperature.—The weekly mean soil temperatures for comparative weeks of the experiment for all 3 plots are plotted in Figure 5. The same thing is found here as in the comparative air temperatures except that the differences are more distinct; Plot 1 had much lower temperatures during the first half of the experiment and much higher during the latter half Plots 2 and 3 had very similar soil temperatures.

All seed potatoes used in these plots were of the Bliss Triumph variety grown in the dry land section of western Nebraska, and were very free of all diseases, having been selected from field inspections the previous year. All seed-pieces were cut to approximately the same size. The seed was all treated with mercuric chloride. Stock cultures of *Fusarium oxysporum* No. 8, 35, and 56 were used for all inoculations. Spore suspensions for inoculating purposes were made from 14-day-old cultures on rice and hard potato agar.[1] Three different methods of inoculations were used. In Method No. 1, the seed tubers were inoculated by boring a hole in the tuber 1 to 1.5 cm. deep and 3 mm. in diameter within 1 inch of a sprout. Small glass tubes of 3 mm. bore and with one end closed were filled with 0.5 c. c. of spore suspension and placed in the cavity of the tuber. The tubers were then planted immediately. In Method No. 2, the seed tubers were inoculated by immersing the freshly cut seed-pieces in a spore suspension of the organism and planted immediately. In Method No. 3, the cut seed-pieces were allowed to callous over for 3 days before

[1] Previous to this experiment these cultures were all tested for virulence by inoculations of healthy tubers in the laboratory. Complete rot of the tubers was obtained at 25°C. in moist chambers.

inoculating as in 2. All inoculations in Plot 1 were made at the same time and with the same spore suspensions. The same was true of all inoculations in Plots 2 and 3. Plot 1 was planted April 20 and dug August 3 to 8. Plots 2 and 3 were planted May 20 and dug September 21. The difference in date of planting was naturally due to the different seasons in the 2 localities.

Plot 1.—Lincoln, Nebraska. This plot contained 9 inoculated rows and 6 control rows, each row having 35 plants. The 9 inoculated rows consisted of 1 row each, for each of the 3 methods of inoculation and each of the 3 strains of *Fusarium oxysporum* used. This plot was examined several times a week thruout the period of the experiment and notes were made when any characteristic differences appeared in the plants. Only 2 plants wilted out of the 315 grown from inoculated seed-pieces. One of these plants was with Inoculation Method 2, Strain No. 56, and the other with Inoculation Method 3, Strain No. 56. All the control plants remained healthy. There was considerable yellowing of the lower leaves and tipburn of all the plants including controls due to the high temperatures.

In this plot the characteristic growth thruout the season was considerably different from that found in Plots 2 and 3. All the plants were slightly dwarfed, the leaves small, wrinkled, or curled, sometimes with a slight mottling, the appearance being very similar to the usual mosaic symptoms but in this case being due entirely to environmental conditions.

The results at digging time are shown in Tables 13 to 18 and are compared with the results found in Plots 2 and 3. Each plant was examined separately, the stems being examined for rotting and for vascular discoloration and the new tubers for vascular discoloration. Many of the plants were mature and the stems had dried out too much to observe infection accurately; these are listed in the tables as "dead."

Plot 2.—Dry land plot at Mitchell, Nebraska. The seed was inoculated and planted in the same way as in Plot 1, except that 33 hills were planted with each of the 3 strains and each of the 3 methods of inoculation. The distance of these plots from the Agricultural College made it impossible to see these fields during the summer more than 3 times. Notes taken at these times were also supplemented by observations made at various times thruout the season by Clarence Harris of the United States Department of Agriculture, stationed at Mitchell. This plot showed the greatest amount of wilt. The results are tabulated in the following tables.

TABLE 13.—*Percentage of plants wilted*

Strain of *Fusarium oxysporum*	Method of inoculation	Plot 1	Plot 2	Plot 3
		Per cent	*Per cent*	*Per cent*
8	1	0	3.0	10.0
	2	0	78.5	14.2
	3	0	69.6	10.0
35	1	0	0	3.1
	2	0	69.6	3.3
	3	0	13.7	3.1
56	1	0	9.0	6.8
	2	2.8	3.5	0
	3	2.8	7.1	14.2
	Control......	0	0	0

Plot 3.—Planted in the same way and at the same time as Plot 2. This plot was irrigated twice. The plants were very vigorous thruout the experiment.

Results.—The percentage of germination was higher in Plot 1 than in Plot 2, and Plot 3 showed the lowest percentage of germination. However, as the controls showed a lower percentage than the inoculated rows in Plots 2 and 3, no conclusions can be drawn as to the effects of inoculations upon germination. Therefore, in the following tables and discussion, the actual number of plants is used as the basis for calculating percentages, rather than the number of seed-pieces planted.

Considering the actual wilting of the plant, i. e., wilting resulting either in death or in wilting from which the plant had not recovered before digging, we find the following percentages in Table 13. This shows that practically no wilt occurred in Plot 1. Plot 2 had the greatest amount of wilt. Strains No. 8 and No. 35 appeared more virulent than No. 56 in Plots 2 and 3. In general, the most successful methods of inoculation were Methods 2 and 3, that is, dipping the seed-pieces in a spore suspension.

Comparing now the resulting tubers from the plants in Plots 2 and 3 where wilting occurred, by examining all tubers at digging time and classing as infected all tubers showing distinct vascular discoloration or stem-end rotting, we find in weight comparisons, which were found to be more accurate than the number of infected tubers, the percentages presented in Table 14.

TABLE 14.—*Comparative percentages of infected tubers by weight, from wilted plants and healthy plants of inoculated rows and healthy plants from control rows*

		Inoculated rows		Control rows
		Wilted plants	Healthy plants	Healthy plants
Plot 2	No. of plants	79	166	138
	Percentage of infected tubers by weight	85.9	33.7	5.3
Plot 3	No. of plants	19	220	144
	Percentage of infected tubers by weight	24.4	15.5	0.4

Thus, the number of tubers having either vascular discoloration or rotting can be correlated not only with wilt but also with apparently healthy plants grown from inoculated seed. The amount of infection was also much greater in Plot 2 than in Plot 3.

If we now compare the total yield from the rows having wilted plants in Plots 2 and 3 (as shown in Table 13), we find

TABLE 15.—*Comparative yields in grams per plant of control rows and wilted and healthy plants in inoculated rows*

		Plot 2	Plot 3
		Grams	*Grams*
Inoculated rows	Wilted plants	134	500
	Healthy plants	244	682
Control rows	Healthy plants	369	973

that the total yield per plant can be correlated, not only with wilted plants, but also with healthy plants from inoculated seed. These results are tabulated in Table 15. The higher yields in Plot 3 where the percentage of infection was low was particularly noticeable. However, the cultural conditions must be taken into consideration in comparing yields in different plots, and of course Plot 3 would have a much higher yield, due to irrigation. The comparison between the different lots of plants within Plot 3 is, however, nearly as marked as in Plot 2.

If we now use for a comparison the number of stems either dead or showing vascular discoloration, we find the following percentages:

		Plot 2	Plot 3
Inoculated rows...	Wilted plants	93 per cent	94 per cent
	Healthy plants	88 per cent	83 per cent
Control rows......	Healthy plants	32 per cent	13 per cent

The same relation holds here as in the table, where the infected tubers were used as a basis of comparison. The percentages, however, were all higher and the difference between inoculated and controls was not as great. This was chiefly due to the fact that many of the stems necessarily listed as dead had matured normally; also the differences between the plants in Plots 2 and 3 are chiefly due to the fact that the plants remained green longer under the irrigated conditions. This index, therefore, is not a very reliable one in judging the amount of disease.

Disregarding wilting as the chief symptom of the disease and comparing the several experimental plots simply on the basis of inoculated seed-pieces as compared with control rows, we find several interesting comparisons.

Table 16 shows the percentage by weight of total tubers showing vascular discoloration or rotting in the various plots.

This table shows that by far the greatest amount of infection took place in Plot 2. The next largest was in Plot 3, except in case of Inoculation Method 2 with *Fusarium oxysporum* No. 56, which was greatest in Plot 1. However, the total of all three types of inoculation with Strain No. 56 was greatest in Plot 2. The amount of infection in Plot 1 was very small, except with Inoculation Method 2, the other inoculations often running lower than the controls, which average well in Plots 1 and 2 and were much lower in Plot 3.

It is also seen that with reference to the strain of *Fusarium oxysporum* used more infected tubers resulted with inoculations

TABLE 16.—*Percentage by weight of resulting tubers having vascular discoloration or stem end rot*

Strain of *Fusarium oxysporum*	Method of inoculation	Plot 1	Plot 2	Plot 3
		Per cent	*Per cent*	*Per cent*
	1	1.2	16.1	0.3
8	2	18.2	85.1	35.7
	3	0.0	88.1	63.4
	1	0.6	11.9	1.3
35	2	10.8	77.6	32.7
	3	2.7	62.7	12.2
	1	2.3	11.8	3.7
56	2	34.0	25.9	3.7
	3	12.0	29.6	4.0
	Control.......	5.0	5.1	0.4

with Strain No. 8 except in Plot 1, where the amount of infection was small and the probable error would be correspondingly greater,—this in spite of the fact that Strain 8 is the oldest strain of the three. As regards the method of inoculation, there was uniformly more infection obtained with Inoculation Methods 2 and 3 than with 1. There does not appear from these results to be a great advantage in allowing the cut surfaces of the seed-piece to callous over before planting.

If, instead of comparing the amount of infection by the total weight of tubers, we compare the number of plants having infected tubers, the following results are found in Table 17.

This table shows the same comparative results as those found in the previous tables, the order of greatest infection being Plot 2, Plot 3, and Plot 1 respectively, except with inoculations with No. 56, where there was more infection in Plot 1 than in Plot 3. It was also noticeable that in general the highest percentages were found with Inoculation Method 2, as in the previous tables. There was no clear correlation between the amount of infection in the different plots and the strain of organism used.

TABLE 17.—*Percentage of mature plants having infected tubers*

Strain of *Fusarium oxysporum*	Method of inoculation	Plot 1	Plot 2	Plot 3
		Per cent	*Per cent*	*Per cent*
8	1	2.7	12.1	3.7
	2	20.0	92.0	57.1
	3	0.0	87.8	86.6
35	1	2.7	27.2	3.1
	2	5.5	85.5	70.0
	3	13.8	79.3	32.2
56	1	11.1	24.2	6.8
	2	34.2	46.4	18.5
	3	30.5	39.2	14.3
	Control.......	10.9	8.8	1.3

Averaging the total number of infected tubers in all 3 methods of inoculations, we find that in Plot 1 the greatest infection, 30 per cent, was with Strain No. 56; in Plot 2, 67.4 per cent, with Strain No. 35; and Plot 3, 50.5 per cent, with Strain No. 8.

The effect of these inoculations on the actual yield of tubers is shown in Table 18, the results being given in weight per plant rather than in total weight for the row, because many plants did not germinate and yet this failure to germinate could not be correlated with the disease.

This shows a higher yield from control plants than from any of the rows grown from inoculated seed, the averages being higher thruout, and only one row (in Plot 3) yielding higher than the control. In general the lowest yields in each plot were found in the rows having the greatest amount of disease as indicated by wilt or by infected tubers. This does not hold true, however, for each individual row when the variation in the amount of disease is small.

At the time this experiment was completed it was impossible to make cultural studies from all the infected tubers. A repre-

TABLE 18.—*Average weight of tubers in grams per plant*

Strain of *Fusarium oxysporum*	Method of inoculation	Plot 1	Plot 2	Plot 3
		Grams	*Grams*	*Grams*
8	1	99	86	723
	2	170	121	658
	3	200	166	383
35	1	175	316	751
	2	161	169	743
	3	186	233	1,133
56	1	185	302	599
	2	94	318	868
	3	168	296	582
Average of	inoculations....	159	234	715
	Controls......	241	369	973

sentative lot of 83 tubers was, however, selected from the various plots, and isolations were made from these, usually 3 isolations being made from each tuber. *Fusarium oxysporum* was recovered from 11 tubers, Fusarium spp. exclusive of *F. oxysporum* from 17 tubers, miscellaneous fungi from 3, and 52 tubers failed to yield any organism in culture. From 36 tubers having varying degrees of vascular discoloration in the absence of stem end rot, *F. oxysporum* was isolated only twice, Fusarium spp. twice, and 128 cultures from the remaining 32 tubers were sterile. From 42 tubers showing both stem-end rot and vascular discoloration, *F. oxysporum* was isolated from 9 of them, Fusarium spp. from 12, miscellaneous fungi from 3, and cultures from 18 tubers remained sterile. Five tubers of this lot showing stem-end rot but no vascular discoloration yielded in culture Fusarium spp. from 3, and bacterial and miscellaneous fungi from 2. While the number of tubers examined was comparatively small, the results were well in accordance with hundreds of other isolations made at various times by the writer.

DISCUSSION

The infection of the plants through the seed-piece from the soil was shown by MacMillan (1919) to be the chief method of infection under irrigated conditions of Colorado. Such infection in the fields in Michigan is not common in the author's experience. It would be assumed that such a type of infection would be more prevalent in the presence of high soil moistures. The results of the field experiments recorded here do not, however, prove this contention to be true. Judging the amount of infection either by the number of wilted plants, the vascular discoloration of the stem, of the tuber, or by comparative yields, it was found that under the 3 widely different environmental conditions present in these experiments the best development of the disease was in the dry land plots of western Nebraska. Comparing this plot (No. 2) with the other plots as to environmental conditions, we find that the soil moisture was less during the entire experiment than that in Plot 1, where the smallest amount of disease developed, and that there was also a much smaller amount of soil moisture than in the irrigated plot (No. 3) during the latter half of the experiment. The corresponding temperatures both of soil and of air were relatively higher than in Plot 1 during the earlier part of the experiment and lower during the later part.

Just what interpretation can be placed on these results is not entirely clear, as the evidence presented here is only for one year. It is evident in these experiments as in all the others reported in this paper that the amount of infection secured by artificial inoculations with pure cultures is so small that it is dificult to make a definite and clear-cut interpretation of the results. No effect was observed on the germinating power of the seed due to inoculation.

RELATION OF ENVIRONMENT TO THE DISEASE

Fusarium oxysporum is capable of living in the soil saprophytically or of overwintering in the seed tuber under a variety of conditions. It is only capable of producing severe effects on its host plant when conditions are very favorable for the infection and progress of the disease. These conditions made up of the complex relations of many factors may determine not only whether infection will take place, but also the method of infection. Conditions favorable for infection often retard the further progress of the disease, and different combinations of environmental factors will cause a marked change in the symptoms.

References in the literature on the relation of climatic factors to the disease, while abounding in incomplete observational data, are rather meagre in exact meteorological records in relation to the various manifestations of the disease. Information regarding soil temperatures and moistures is regrettably lacking. Nevertheless many conclusions are set forth as to the effect of different factors upon the disease. Many of these are misleading owing to the incompleteness of the data and to the casual consideration of the disease *in toto*, rather than to a close comparison of the successive stages in its development in relation to the environmental factors.

Fusarium wilt has always been considered to be a disease favored by warm climates, and some authors have held that a combination of high temperature and drought is particularly favorable for its development. While it is true that the disease is not reported as being serious in some of the northern potato producing states, yet considerable infection was found by the writer in the upper peninsula of Michigan in 1914 and 1915. In Nebraska the disease is much more prevalent in the western section of the State with an altitude about 3,500 feet higher and a correspondingly cooler temperature, than in the eastern part of the State. Furthermore, we find that such generalizations do not hold when we examine individual fields, experimental plots, or sometimes even potato growing sections of considerable size.

Manns (1911) observed that drought apparently hastened the yellowing symptoms of the disease, but he was unable to decide its influence upon the activity of the organism. He states (p. 317) that in the history of the disease at the Ohio station—

> the severest attack occurred during the most favorable season we have had in a long time, viz., in 1909. The temperature and rainfall that season were very equitable.

He believed (p. 317) that as far as reducing the yields was concerned the disease would cause—

> as great a percent in reduction under favorable conditions as under drouth.

He claimed, however, that the disease would penetrate much deeper into the tubers under conditions of drought.

MacMillan (1919) stated (p. 209):

> Temperatures of the soil are vital as regards infection. The critical temperature for infection has not been determined and it varies for the manner of infection. Seed-piece infection will occur at a considerable lower temperature than root infection.

Bisby (1920), observing the prevalence of "foot-rot" in rela-

tion to climatic factors, finds that "foot-rot" develops only when abundant moisture is present, even tho the temperature may be considerably below normal, and that it occurs only late in the season, irrespective of whether the conditions have been favorable from planting time on.

Haskell (1919) finds that soil temperature is the chief factor in influencing the disease, and states (p. 258) that—

> the disease is most severe in places where high summer temperatures prevail at the time when tuber formation is in progress * * * Within Dutchess County there is a distinct correlation between the amount of disease and factors influencing soil temperature such as altitude, exposure of fields and shading of plants.

He had difficulty in obtaining infection with artificial inoculations unless a high soil temperature, 36° to 40°C., was used.

Edson and Shapovalov (1920) report an experiment in which seed tubers inoculated with *Fusarium oxysporum* were planted at two different dates. The experiment was conducted in the vicinity of Washington, D. C. They conclude that—

> there were more tubers infected with *Fusarium oxysporum* in the early crop grown at higher temperature, than in the late crop grown at lower temperature.

They give no detailed information regarding temperature and moisture conditions during the experiment.

Experiments made by the author and discussed elsewhere in this paper show results very similar to those reported by Manns (1911). For instance, in working with infected seed tubers, the disease was reproduced abundantly in Michigan in 1915, a season with low temperatures and high precipitations; while in 1916 similar experiments yielded negative results when the temperatures were extremely high and precipitations very low, in fact, one of the hottest and driest seasons on record for Michigan. Inoculation experiments also gave negative results during this season.

Experiments with inoculated seed in Nebraska have shown that the disease is favored by the environmental conditions found in the dry land sections of western Nebraska more than in either the irrigated fields having a higher soil moisture or the fields of eastern Nebraska with both higher temperature and moisture during the entire season. In the experiments under control conditions, low soil moisture during the early growth of the plants was unfavorable for infection. However, high soil moisture followed by drought resulted in an increase in the amount of wilt, while continued high moisture decreased the percentage of wilt even tho the extent of rotting of the stem and roots was increased.

In the temperature experiments, plants infected at high temperatures and changed to lower temperatures recovered and showed no wilt symptoms even tho the organism was present in the host tissue. Plants started at low temperatures and later changed to higher temperatures showed as much disease as those grown at a maintained high temperature.

Soil moisture may act in a variety of ways, determining the distribution of the organism thru the soil, and by its effect on the host plant determining the entrance of the organism. Later changes in moisture not only would have a profound effect upon the type of injury, i. e., rotting of the stem or roots, but would also affect the symptoms of the disease by bringing about conditions enabling the host plant either to counteract the detrimental effects of the organism or to accelerate the action of the parasite and weaken the host.

Temperature would affect not only the growth of the organism but also the growth of the host and determine the ability of the organism to enter the host. The conditions favorable for this infection may not be favorable for the further progress of the disease within the host plant and the susceptibility of the host to succumb to the attack of the parasite.

Because of these complex reactions we often find conditions during a greater part of the season that would be favorable for the progress of the disease, but unless the conditions had been favorable for the inital infection, these later environmental conditions would have no effect. Conditions of drought, while favoring the later stages of the disease, are apparently not favorable for infection. Drought combined with high temperatures may cause a browning of the vascular system in the absence of the parasite, a condition often mistaken for Fusarium wilt.

Thus it is clear that observational data on environmental conditions to be of value must take into consideration all conditions influencing the disease from the time of planting until maturity. It is only by the accumulation of data from all potato growing sections, combined with a careful examination of the plants as regards the method of infection and resulting symptoms, that we can come to a clear understanding of the action of this disease.

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